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The Antidiabetic and Hypolipidaemic Effects of *Cicer arientinum*, *Cinnanomum verum* and *Citrus aurantifolin* in Types I and II Diabetes Mellitus

By

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بسم الله الرحمن الرحيم

قال تعالى: (وفوق كل ذي علم عليم)
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(وما أوتيتم من العلم إلا قليلاً)
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Dedication

I dedicate this research

To the spirit of my Sheikh Professor Hasan Alfatih Qaribullah for choosing the topic of my research and to his Khalifa Sheikh Mohammed for his spiritual support for

To my little family specially my lovely son Ahmed Alhasan and to all members of my big family specially my parents who took the burden of looking after my son in order to release me for my research.

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List of Contents

List of Tables

List of Figures

List of Photos

List of Abbreviations

Alkaline phosphatase	ALP
Alanine amino transferase	ALT
Aspartate aminotransferase	AST
Cicer arientinum	*C. arientinum*
Citrus aurantifolin	*C. aurantifolin*
Cyclocarya paliurus	C. paliurus
Cinnamomum verum	*C. verum*
Ethylene diamine tetra acetic acid	EDTA
Glutamate Oxaloacetate Transaminase	GOT
Glutamate pyruvateTransaminase	GPT
Glycated haemoglobin	A_{1C}
Haemoglobin	Hb
High fat diet	HFD
Insulin dependent diabetes mellitus	IDDM
Momordica cyambalaria	M. cyambalaria
Medicinal and Aromatic Plants Research Institute	MAPRI
Non- esterified fatty acids	NEFAs
Non-insulin dependent diabetes mellitus	NIDDM
Oral Glucose Tolerance Test	OGTT
Origanum vulgare	OV
Red blood cells	RBCs
Revolutions/minute	Rpm
Streptozotocin	STZ
Total red blood cells	T.RBCs
Total white blood cells	T.WBCs
Very low density lipoproteins	VLDL
water ethanol extracts	WEE
Wild Ginseng ethanol extract	WGEE

Abstract

Objectives: This research aimed to study the antidiabetic effect of the aqueous and methanolic extracts of the seeds of *Cicer arientinum*, the bark of *Cinnanomum verum* and the leaves of *Citrus aurantifolin* in adult Wistar Albino rats.

Methodology: Type II diabetes mellitus was induced using intraperitoneal loading dose of glucose while type I was induced using intraperitneal dose of Streptozotocin. Glucose tolerance test was adopted to follow the glucose level after administration of plant extracts. Blood samples for measurement of glucose, cholesterol and triglycerides were collected from the Retro-orbital plexus of the eye using heparinized capillary tubes. For type II diabetes blood samples were collected every one hour for four hours while for type I they were collected every four hours for twelve hours. Glibenclamide was chosen as a reference drug for type II and Insulin was chosen for type I. Furthermore these plants were investigated for their toxicological effects in terms of their actions on liver functions, kidney functions, blood haemoglobin, total white and red blood cells count. Even further, these plants were phytochemically screened for investigation of their chemical constituents

Results: The findings of this research showed that all plants revealed a pronounced hypoglycaemic effect. Concerning type II, the most significant effect ($P \leq 0.001$) was exerted by the extracts of *Cinnanomum verum*, throughout the experiment as compared to the control and to the reference drug Glibenclamide. Concerning type I, the aqueous extract of *Cicer arientinum*, showed an early onset of hypoglycaemic effect which started since the first hour, while *Cinnanomum verum* and *Citrus aurantifolin*

showed a significant glucose reduction (P≤0.001) two and four hours post dosing, as compared to the control and the reference drug Insulin. Concerning the hypolipidaemic effect in type II, the methanolic extract of *Cicer arientinum,* showed a significant reduction (P≤0.001) in the levels of blood cholesterol and triglycerides, throughout the experiment. In type I, *Citrus aurantifolin* reduced blood triglycerides significantly (P≤0.001) since the first hour. The findings of the toxicological study revealed no alteration in the normal liver and kidney functions as well as haemoglobin and blood cell counts. Phytochemical screening of the study plants revealed that alkaloids were found to be common in all of them.

Conclusion: It can be concluded that *C.arientinum, C.verum* and *C.aurantifolin* can be promising antidiabetic drugs with a great safety and no side effects. This verifies the traditional use of these plants as hypoglycaemic agents.

الاطروحة

الأهداف: لقد هدف هذا البحث لدراسة الأثر المضاد لمرض السكرى فى المستخلصات المائية والكحولية لبذور نبات الكبكبيه، لحاء نبات القرفة وأوراق نبات الليمون فى جرذان الويستر البيضاء الناضجة .

منهجية البحث: تم انشاء النوع الثانى من مرض السكرى معمليا باستخدام جرعة مثقلة من السكر بينما أنشئ النوع الأول من مرض السكرى باستخدام مادة الستربيتوزوتوسين فى التجويف البيريتونى للجزذان. وقد اتبع اختبار تحمل الجلوكوز لمتابعة مستوى السكر بعد اعطاء جرعات مستخلصات النباتات. وقد جمعت عينات الدم من كتلة الأوعية الدموية خلف كرة العبن، لقياس مستوى الجلوكوز، الكوليسترول والدهنيات الثلاثية، وذلك باستعمال انابيب شعرية تحتوى على الهيبارين.بالنسبة للنوع الثانى من مرض السكرى فقد جمعت عينات الدم كل ساعة لمدة أربعة ساعات بينما جمعت عينات الدم للنوع الأول كل أربعة ساعات لمدة اثنى عشر ساعة. وتم اختيار القليبنكلامايد كدواء قياسى مرجعى للنوع الثانى من مرض السكرى بينما أختير الانسولين كدواء قياسى للنوع الأول. و اضافة الى ذلك فقد تم استكشاف أثر السموم فى هذه النباتات عن طريق دراسة أثرهم على وظائف الكبد، وظائف الكلى، الهيموقلوبين، عدد كريات الدم البيضاء والحمراء وأكثر من هذا فقد تم مسح عام للمكونات الكيميائية لهذه النباتات.

النتائج: وأوضحت النتائج أن كل النباتات أفصحت عن أثر واضح فى خفض مستوى سكر الدم. أما نالنسبة للنوع الثانى فقد أحرز مستخلصى نبات القرفة الأثر الأكثر أهمية احصائية ($P\leq0.001$) فى خفض مستوى سكر الدم طوال فترة التجربة مقارنة مع مجموعة الضبط والدواء المرجعى القليبينكلامايد. أما بالنسبة للنوع الأول من مرض السكرى، فقد أوضح المستخلص المائى لنبات الكبكبه مفعول مبكر فى خفض مستوى السكر فى الدم واذى بدأ منذ الساعة الاولى، بينما أوضح نباتى القرفة والليمون انخفاض ذو أهمية احصائية ($P\leq0.001$) فى مستوى السكر فى الساعة الثانية والرابعة بعد تعلطى جرعات المستخلصات، مقارنة مع مجموعة الضبط والدواء المرجعى الانسولين. أما بالنسبة لأثر هذه النباتات فى خفض مستوى الدهون فى الدم لدى النوع الثانى من مرض السكر. فقد أوضح المستخلص الكحولى لنبات الكبكبيه انخفاض ذو أهمية احصائية ($P\leq0.001$) فى مستوى الكوليسترول والدهون الثلاثية طوال فترة التجربة. أما بالنسبةللنوع الأول من مرض السكرفقد خفض مستخاصى نبات اليمون، الدهون الثلاثية انخفاض ذو أهمية احصائية

($P \leq 0.001$) منذ الساعة الأولى. اما فيما يتعلق بالدراسة السمية لهذه اللنباتات فلم توضح الدراسة أى تغييرفى المستوى الطبيعى فى كل من وظائف الكبد، وظائف الكلى، الهيموقلوبين، عدد كريات الدم البيضاء والحمراء . اما فيما يتعلق با لمكونات الكيميائية لنباتات الدراسة وجود مادة الألكالويدس كعنصر مشترك فى كل من نبات الكبكبيه القرفة وأوراق الليمون.

الخلاصة: يستخلص من هذه الدراسة أن نباتات الكبكبيه، القرفة والليمون يمكن أن تكون أدوية واعدة مضادة لمرض السكرى، ذوات مأمونية عالية و ليس لديهم أثار جانبية. هذا يؤيد الاستعمال البلدى لهذه النباتات كعوامل خافضة لمستوى السكر فى الدم.

Chapter One

Literature Review

1. Introduction:

The use of herbs and medicinal plants in treatment of different diseases is as old as man himself. Mankind in ancient ages depended totally in treatment of diseases by herbs and medicinal plants, but this vanished gradually with urbanization of life-style except in very few areas of the world. With the spread of civilization, herbs and medicinal plants were gradually replaced by drugs and medicines of a chemical nature. With time a majority of recent drugs exfoliated many undesirable side effects and severe complications which may be more dangerous than the target disease itself. Furthermore, chemical drugs are relatively more expensive. Such disadvantages of modern drugs, caused mankind to refer again to the old use of herbs and medicinal plants in treatment of a variety of diseases including diabetes mellitus. For a long time, diabetes have been treated orally with several medicinal plants (Aktar and Ali 1984).Synthetic hypoglycaemic agents can produce serious side effects and are not suitable for use during pregnancy (Laner 1985). Therefore, the search for more effective and safer hypoglycaemic agents has continued to be an important area of active research .Investigations on hypoglycaemic agents from medicinal plants has become more important (WHO expert committee, 1980).

Being a very large country with different climatic conditions, ranging from very dry deserts to very damp forests, Sudan is very rich in a vast variety of medicinal plants. Many Sudanese tribes , specially the western tribes , depend almost totally on herbal treatment for many diseases including diabetes mellitus , which is one of the most famous

and widely spreading diseases . The records conducted by the National Center For Health Statistics in 1996 revealed that 0.3% of the population of Sudan are suffering from diabetes mellitus with mortalities reaching up to 4.1% .This is encouraging to researchers to undergo studies in this field , so as to confirm and develop such traditional treatments . From the list of Sudanese hypoglycaemic plants, *Cicer arientinum* and *Citrus aurantifolin* are chosen for this study in addition to *Cinnamomum verum* which is an Indian plant. They are recorded as traditional hypoglycaemic agents but no pervious scientific studies were found to justify

1.1. Some world- wide reviewed hypoglycaemic medicinal plants:

Acasia arabica

Aloe vera

Anacardium occidentale

Artemisia dracuncuus

Artemisia herba alba

Azadirachta indica

Baphia nitda

Bidens pilosa

Boerhaavia diffusa

Caralluma edulis

Carum carvi

Catharanthus roseus

Cissus sicyoides

Coccinia indica

Cogniauxia podoleana Baillon

Cyclocarya paliurus

Eucalyptus globules

Eugenia jambolama

Ferula persica

*Fraxinus excelsio*r

Gongronema. latifolim

Guazuma ulmifolia,

Gulnar farsi,

Gum alibanum,

Gum assafooetida

Gymnema sylvestre

Hydrastis Canadensis

Inula racemosa,

Kola acuminata,

Lepechinia caulescens, ,

Loranthus micranthus

Marrubium vulgare,

Mominda lucida

Momordica charantia

Momordica cyambalaria

Musa sapientum,

Nigella sativa

Ocimum sanctum

Opuntia Streptacantha

Paronychia argentea,

Psacalium peltatum

Punica granatum

Rhizophora mangle

Rubus imperialis

Sclerocarya birrea

Scoparia dulcis

Silybum marianum

Tinospora cordifolia

Tinospora crispa

Trichosanthes kirilowi,

Trigonella foenum graceam

Tuner diffusa

Viscum album

Zizyphus mauritiana

Zizyphus sativa

Table(1-1) Sudanese Hypoglycaemic Medicinal Plants

Arabic Name	English Name	Latin name	Family	Habitat	Chemical constituent	Folkloric Use
الحنظل	Bitter apple, Vine of sodon	*Citrullus colocynthis*	Cucurbitaceae	Wide spread in low lands	Glycosides, flavonoides, alkaloids and saponin	Treatment of dermatitis, eczema, loss of hair,T.B. and **diabetes mellitus**
الدمسيسة	Damsisa	*Ambrosia maritime*	Asteraceae	North and central Sudan	Protein, carbohydrates, fat, and amino acid.	Nephritis, renal calculi. The leaves are used for treatment of **diabetes mellitus**
الشعير البلدى	Barley	*Hardeuvulgare L.*	Loaceae	Various areas	Anthraquinone and Barbaloin	For treatment of calculi. and **diabetes mellitus**
الصبار	…… ….	*Aloe Spp*	Liliaceae	Desert	Volatile oil, Sulpher compounds, vitamins and peptides.	Removal of pyrexia, pains of colon and for treatment of **diabetes mellitus**
التوم	Garlic	*Allium sativa L.*	Liliaceae	Various areas	Glycosides,flavonid -es, steroids and saponin	Treatment of hyper-tension, wounds cholesterol and **anti diabetic**
الحرجل	Argel	*Solenostemma argel*	Asclepiadaceae	low lands Wide spread in Northern Sudan	Protein, carbohydrates, fats, amino acid Volatile oil, alkaloids and saponins.	Treatment of colic, **diabetes mellitus** and locally for treatment of measles.
الحلبة	Fenugreek , Greek	*Trigonella foenum-*	Fabaceae	Various areas	flavonoides,alkaloid s, saponin and	For treatment of

	clover	*graecum L.*			vitamins	abdominal pain, diarrhoea, dysentery and **diabetes mellitus.**
البرسيم	Alfa alfa	*Medicago sativum*	Papilionoideae	Various areas	Rotenone,coumarin, glactoside and steroids	Renal calculi and **diabetes mellitus**
الهجليج	Desert date	*Balaniteae egyptica*	Balanitaceae	low lands	Glycosides and flavonides	For treatment of **diabetes mellitus**
الكلكل	Kulkul	*Bauhinia rufescens*	Caesplpiniacecea -e	low lands	Glycosides and flavonides.	The maceration of the leaves used for treatment ofD.mellitus **diabetes mellitus**

1-2-**Diabetes mellitus:**

Diabetes mellitus is a clinical syndrome characterized by hyperglycaemia due to absolute or relative deficiency of insulin (Christopher, *et a*l 2002).The hyperglycaemia is usually accompanied by glycosuria (Whitby, *et al* 1990).

1-2-1-Epidemiology:

Diabetes is world-wide in distribution and its incidence is rising. In the year 2000, 150 million people world-wide had diabetes, and this is expected to double by 2010. This involves type II principally (Christopher-H, *et al*; 2002). It is estimated that over 13 million people in the United States suffer from diabetes and that 650,000 new cases are diagnosed every year. Most diabetics (over 90%) have non-insulin dependent diabetes mellitus (NIDDM) or type II (Burke, *et al*., 1999)

1-2-2-**Predisposing Factors:**

Increased longevity, obesity, unsatisfactory diet, sedentary lifestyle and increasing urbanaization (Christopher-H, *et al*; 2002).

1-2-3-**Types Of Diabetes mellitus :**

Diabetes manifests itself through abnormally high glucose levels and comes in two major forms:

(a) Insulin dependent (typeI)

(b) Non – insulin – dependent (type II) (Ford and Earl 2002).

1-2-3-1-**TypeI (Insulin dependent diabetes mellitus, IDDM):**

This usually presents acutely in young, non-obese subjects, but it can occur at any age. In general, insulin is required for treatment and ketosis is liable to occur.

1-2-3-2-**TypeII (Non –insulin – dependent diabetes mellitus, NIDDM):**

This type may be subdivided into non-obese and obese categories. It usually presents less acutely than type I, mainly in older (over 40 years

old), obese subjects. Insulin is not required to prevent ketosis as these patients are relatively resistant to the development of ketosis, but it may be needed for correction of abnormalities of glucose (Whitby., *et al* 1990).

1-3-Insulin:

It is a peptide hormone synthesized by the beta cells of the pancreas. Its inactive form, pro-insulin, is formed from A and B chains connected by a connecting peptide (C- peptide). This pro-insulin molecule folds and A and B chains become linked by disulphide bridges forming the active hormone insulin. The beta cells then contain insulin and C – peptide which split off with every unit formation of insulin. Since C-peptide has a longer half – life in plasma than insulin, it is sometimes measured as index of endogenous insulin secretion (Whitby. *et al*1990).

1-4-Normal Glucose Metabolism:

Blood glucose is tightly regulated by homeostasis within a narrow range of 3.5-6.5 mmol/L. (64-118 mg/dl). When intestinal glucose absorption declines between meals, hepatic glucose output is increased in response to counter regulatory hormones glucagons and adrenaline and it falls during prolonged starvation as fats become more important as fuel. The liver produces glucose by gluconeogenesis and glycogen breakdown. Insulin is the only anabolic hormone and it has profound effects on the metabolism of carbohydrates, fats and proteins. It is secreted from pancreatic beta-cells in response to a rise in blood glucose. It lowers blood glucose by suppressing hepatic glucose production and stimulating peripheral glucose uptake in skeletal muscles and fat, mediated by the glucose transporter, GLUT4. Adipocytes and the liver, synthesize triglycerides (from non- esterified fatty acids (NEFAs) and glycerol). Insulin stimulates lipogenesis and inhibits lipolysis, so preventing fat catabolism. Lipolysis, mediated by triglyceride lipase, is stimulated by

catecholamine and liberates NEFAs, whose partial oxidation in the liver provides energy to drive gluconeogenesis and also produce ketone bodies (acetoacetate, which can be reduced to 3-hydroxybutrate or decarboxylated to acetone). Ketone bodies are organic acids which (in small amounts) are oxidized and utilized as metabolic fuels .The rate of utilization of ketone bodies by peripheral tissues is limited, and when the rate of production by the liver exceeds their removal, hyperketonaemia results. Ketogenesis is regulated by the supply of NEFAs reaching the liver and is therefore enhanced by insulin deficiency and release of the counter regulatory hormones that stimulate lipolysis (Christopher., *et al* 2002).

The important metabolic sites that are sensitive to insulin include the liver, where glycogen is synthesized, stored and broken down; skeletal muscles, where glucose oxidation produces energy; and adipose tissues, where glucose may be converted to fatty acids(Christopher., *et al* 2002) (Erict, 1992).

Table (1-2): Metabolic Actions of Insulin

Anabolic Effects (increased)	Catabolic effects (decreased)
Carbohydrate metabolism	**Carbohydrate metabolism**
*Glucose transport (muscle and adipose tissues) *Glucose phosphorylation *Glycogenesis *Glycolysis *Pyruvate dehydrogenase activity *Pentose phosphate shunt	*Gluconeogenesis. *Glycogenolysis.
Lipid metabolism	**Lipid metabolism**
*Triglyceride synthesis. *Fatty acid synthesis (liver). *Lipoprotein lipase activity (adipose tissues).	*Lipolysis. *Lipoprotein lipase (muscle).
Protein metabolism	**Protein metabolism**
*Amino acid transport *Protein synthesis	*Protein degradation.*

1-2-4- Metabolic Disturbances in Diabetes:

Insulin deficiency causes disturbance in carbohydrate, lipid and protein metabolism:

1-2-4-1- Carbohydrate metabolism:

Insulin deficiency leads to decreased glucose uptake by tissue**,** decreased glucose oxidation, increased gluconeogenesis and increased glycogenolysis. (Whitby. *et al*1990)

1-2-4-2- Protein metabolism:-

Anorexia and vomiting in uncontrolled diabetes interfere with orderly replacement in the daily turn over of body pool. Excess plasma cortisol, exert protein catabolic effect and interfere with amino acid incorporation to protein and thus become the source of gluconeogenesis, thereby contributing both to the hyperglycemia and ultimate loss of nitrogen from body (Ahuja 1983).

1-2-4-3- Disturbance in fat metabolism:-

Adipose tissue is most sensitive to insulin action therefore low insulin activity is capable of suppressing fat storage and enhancing lipolysis .Fatty acid liberated from lipolysis in addition to being metabolized by liver in to ketone bodies ,can also be re-esterified and packaged in to VLDL .Further more, insulin deficiency cause a decrease in lipoprotein lipase, the enzyme responsible for hydrolysis of VLDL triglycerides in preparation for fatty acids storage in adipose tissue **(**Christopher., *et al* 2002**).**

1-2-5- Diagnosis Prior to 1997 diabetes was suspected if an individual had at least two fasting glucose readings of 140 mg/dl (7.8 mmol/L) on two separate occasions. In 1997 the American Diabetes Association lowered the diabetes cut-off point to 126 mg/dl (7 mmol/L) on two separate occasions. So now the rules are:

- Fasting glucose level less than 110 mg/dl is considered normal

- Fasting glucose level between 110 and 126 mg/dl indicates impaired glucose tolerance (insulin resistance)

Fasting glucose level at or above 126 mg/dl indicates diabetes**, (**Expert committee 2002)

1-2-5-1-Indications for oral glucose tolerance test:

.Fasting plasma glucose 6.1-6.9 mmol/l.

.Random plasma glucose 7.0-11.0 mmol/l.

N.B. HbA_{1C} is not for diagnosis.

1-2-5-2-Oral Glucose Tolerance Test (OGTT)

a) Fasting plasma sample is taken after overnight fast (8-12 hours).

b) Then patient is given 75 grams glucose orally.

c) Plasma and urine glucose concentrations are determined in fasting and at 30 minutes intervals for 3 hours after glucose ingestion. (Whitby. *et al*1990).

1-2-5-2-1- Interpretation of the results of OGTT:

- 2-hour reading of less than 140 mg/dl (7.8 mmol/L) is normal
- 2-hour reading of between 140 and 200 mg/dl is indicative of impaired glucose tolerance
- 2-hour reading of 200 mg/dl or greater is indicative of diabetes.

The National Institutes of Health recommends that diabetes should be suspected only if at least two readings in the 2- hour period are equal to or greater than 200 mg/dl **(**Expert committee 2002). There is evidence that food intake during the two days prior to the glucose tolerance test can materially affect the results. It is best to eat a diet rich in complex carbohydrates and avoid an excessive intake of protein and fats (Whitaker and Julian 2001).

1-5-Glycated haemoglobin (A_{1C}):

Glycated haemoglobin provides an accurate measure of glycaemic control over a period of weeks to months. This can be utilized as an

assessment of glycaemic control in a patient with known diabetes, but is not sufficiently sensitive to make a diagnosis of diabetes and is usually normal in patients with impaired glucose tolerance. (Christopher., *et al* 2002). Glycated haemoglobin is formerly named glycosylated haemoglobin (Whitby. *et al*1990). Haemoglobin A_{1C} is the major haemoglobin in adults (97%)**.** Normally, about 5-8 % of haemoglobin A_{1C} reacts none enzymatically with glucose to form glycated haemoglobin or HbA_{1C}. This remains over the life span of RBCs (120 days). The concentration of glycated haemoglobin is directly proportional to the glucose level over the life span of RBCs (Arise of 1 % in Hb A_{1C} corresponds to an approximate average increase of 2 mmol/l of blood glucose. (Christopher, *et al* 2002). In diabetes mellitus with uncontrolled hyperglycaemia, glycated haemoglobin will be 12 % or more high.

1-6-1-Importance of Hb A_{1C} :

It is used as an index of diabetic control over 2-3 months .It correlates with the mean plasma glucose concentration during this period. The higher the percentage, the poorer the mean diabetic control (Whitby. *et al*1990).

1-2-6- Complications of diabetes:

The most serious problem with high glucose levels is that the excess glucose tends to bind to proteins and cause them to become ‘sticky’. This process is called glycosylation and is a major factor in atherosclerosis and other diabetes complications. The higher than normal insulin levels (hyperinsulinaemia) experienced by people with insulin resistance or type II diabetes is also highly detrimental. They interfere with the normal metabolism of fats and cause an increase in the production of very – low – density cholesterol, a potent risk factor of heart disease. High insulin levels also encourage the formation of blood clots and thereby increase

the risk for heart attacks. Finally, High insulin levels can lead to hypoglycaemia (low blood sugar level).

Long-term, uncontrolled high blood sugar levels can lead to a number of serious complications; among them are kidney failure, hypertension, diabetic retinopathy (eye disease) neuropathy (nerve disease), heart disease, peripheral vascular disease (intermittent claudication), stroke and diabetic foot disease. (Whitaker and Julian 2001).

1-2-7- Guide lines to therapy:-

All patients with diabetes require diet therapy .Good glycaemic control is unlikely to be achieved with insulin or oral therapy when diet is neglected, especially when the patient is also over weight (Christopher., *et al* 2002).

1-2-7-1-Management of Diabetes:

Three methods of treatment are available:

1- diet alone
2- oral hypoglycaemic drugs and
3- Insulin.

All patients with diabetes require diet therapy .Good glycaemic control is unlikely to be achieved with insulin or oral therapy when diet is neglected, especially when the patient is also over weight (Christopher., *et al* 2002).

-Approximately 50 % of new cases of diabetes can be controlled adequately by diet alone.

-20-30 % will need an oral hypoglycaemic drug and

-20-30 % will require insulin.

The ideal management of diabetes would allow the patient to lead a completely normal life.

Diet, exercise and oral hypoglycaemic agents are the mainstay of conventional treatment of insulin resistance and type II diabetes

(Whitaker and Julian 2001).The diet emphasizes the avoidance of simple sugars and an increased intake of fiber (Bernstein., *et al* 1997).

Exercise helps to reduce insulin levels and lowers cholesterol and triglyceride levels as well as blood pressure (Spelsberg., *et a*l 1995). Many patients with insulin resistance or type II diabetes can actually revert to a non-diabetic state just by exercising and following a proper diet (Murray., *et al* 1998).

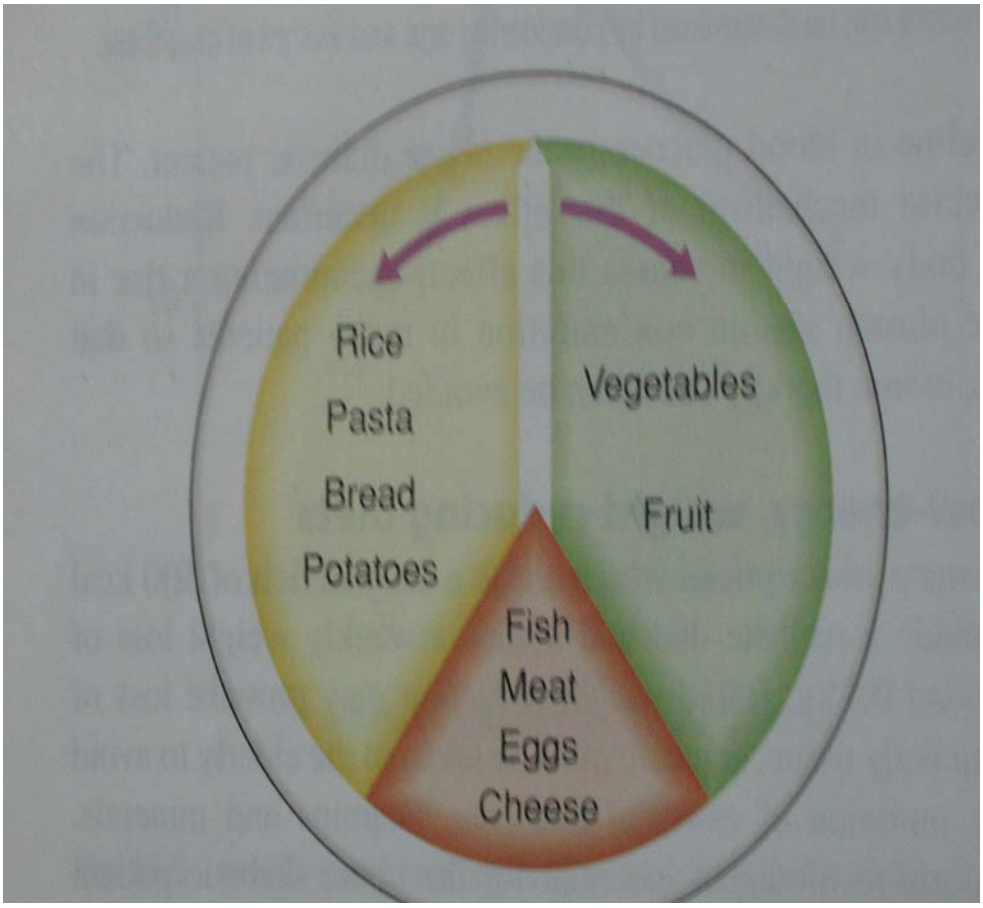
1-2-7-2- Diet for insulin – treated diabetes:

An insufficient dose of insulin for a meal with a large carbohydrate content leads to post – prandial hyperglycaemia, while inadequate carbohydrate consumption risks hypoglycaemia.

A useful meal-planning tool is the plate model which encourages the inclusion of carbohydrate as the main part of the meal in conjunction with vegetables, and limits the consumption of protein – containing foods. (Christopher., *et al* 2002).

1-2-7-2- Diet for insulin – treated diabetes:

Fig. (1-1)



1-2-7-2-1- Oral hypoglycaemic drugs:

There are various, and although their mechanisms of actions are different, most depend upon a supply of endogenous insulin and they therefore have no hypoglycaemic effects in patients with type I diabetes.

1-2-7-2-1-1-Combined Oral Hypoglycaemic Therapy and Insulin:

In diabetic patients who are requiring increasing doses of sulphonylurea or biguanide , either alone or in combination with each other or with a thizolidinedione , the introduction of a single dose of an intermediate – acting insulin (usually isophane), administered at bedtimes may improve glycaemic control and delay pancreatic beta cell failure . The exogenous insulin suppresses hepatic glucose output during

the night and lowers fasting blood glucose. This treatment is ineffective in diabetic patients who have no residual endogenous insulin i.e. (C-peptide –negative). For patients who are approaching secondary failure to oral medication, this provides a single and effective introduction to self-treatment with insulin with little risk of hypoglycaemia (Christopher., *et al* 2002).

Table (1-3): Oral Hypoglycaemic Agents

Drug	Mechanism of action	Indications	Dose	Side effects
Sulphnylureas Tolbutamide, Chlorpropamide Glibenclamide , Glimepiride	**.**Stimulate release of insulin (insulin secretagogues). **.**reduce hepatic gluconeogenesis.	For non –obese patients with type II diabetes.		Increase in weight.
Biguanides Metformin	**.**increase insulin sensitivity and peripheral glucose uptake. **.**impairs glucose absorption by the gut.	For obese patients as it is not associated with a rise in body weight.	500-1 g, (12-hourly).	GIT symptoms. **Contraindicated:** In patients with impaired renal or hepatic function and in alcohol addicts.(risk of lactic acidosis)
Alpha-Glucoside Inhibitors Acarbose, moiglitol.	**.**delay carbohydrate absorptions in the gut by selectively inhibiting disaccharides. **.**lower post-prandial blood glucose **.**improve overall glycaemic control.	Good for obese patients.	Taken with each meal.	Flatulence, abdominal blooting and diarrhoea.
Thiazolidinedion (TZD) Glitazone , Rosoiglitazone, Pioglitazone	**.**enhance the action of endogenous insulin (mainly in adipose tissue).	Indicated for patients with insulin resistance.	Should be prescribed with either a sulphonyl ureas or metformin	Weight gain and fluid retension. **Contraindicated:** In people with cardiac failure.
Meglitinides Repaglinide	**.**regulate prandial glucose.	For non obese patients.	Immediately before	Increase body weight.

Nateglinide.	.stimulate endogenous insulin secretion		food and prescribed with metformin	

1-2-7-2-1-2-Side effects of some oral hypoglycaemic drugs:

Sulphonylureas have the potential for many quite serious side effects. Among the more common are hypoglycaemia, weight gain, gastrointestinal problems, and hypothyroidism and skin rashes. They also increase the risk of heart disease and circulatory problems (Whitaker and Julian 2001).

Metformin (Glucophage) and thiozolidinediones, have the potential for some very serious side effects such as increase in blood pressure, increase in heart diseases, lactic acidosis, weight gain, increase in low – density – cholesterol and liver damage (Whitaker 2001).

The potentially devastating side effects of these drugs can be completely avoided by replacing them with natural supplements (Whitaker 2001).

1-2-3-6- Experimental diabetes:-

Specific cytotoxic agents such as alloxan or streptozotocin can selectively destroy the β-cell of the pancreas. Injection of these agents produces a form of chemical diabetes, which closely resembles diabetes due to surgical removal of the pancreas. Streptozotocin is abroad-spectrum antibiotic with anti-tumor and oncogenic properties which is widely used as a diabetogenic agent in experimental animals..This action is mediated by beta-cell destruction, which results in the development of an insulin-dependant syndrome (Rakieten, et al; 1963 and Herr., *et al* 1967).

1-2-3-7- A Survey on the anti-diabetic activity of some medicinal plants:

Recently, many experimental and clinical trials were adopted to detect the hypoglycemic and anti diabetic effects of many medicinal plants used in the folk medicine for the treatment of diabetes mellitus.

1-2-3-7- 1- Experimental studies on laboratory animals

Acetone extraction of seeds of *Trigonella foenum graceam* exhibited a dose related hypoglycaemic effect on cadmium or alloxan – induced hyperglycaemic rats. The induced hyperglycaemia was antagonized by the acetone extracts of the seed, stem and leaves of *Trigonella foenum*. These extracts appear to act at the cellular level to produce hypoglycaemic effects on normal and cadmium or alloxan – induced hyperglycaemic rats. (Ghafghazi., *et al* 1977).

Among a mixture of five plants in used by Kwaiti diabetics only the Myrrh and aloe gums effectively increased glucose tolerance in both normal and diabetic rats. The remaining components, *Gum alibanum, Nigella sativa* seeds and *Gum assafooetida* were without effect (Al-Awadi and Gumaa 1987).

Petroleum ether extracts of Inula racemosa, lowered plasma insulin and glucose levels within 75 minutes of oral administration to albino rats and it significantly counteracted adrenalin –induced hyperglycaemia. All these findings indicate that one of the constituents of *Inula racemosa* may have adrenergic beta blocking activity (Tripathi., *et al* 1988).

Oral administration of an aqueous extract of the aerial parts of *Artemisia herba alba* to normoglycaemic and to alloxan –diabetic rabbits, produced significant hypoglycaemic activity which was consistent and time dependent (Twaij and Al Badr 1988).

An aqueous decoction of the aerial part of *Teucrium* hyperglycaemic rats indicated a significant reduction in blood glucose concentration 4 hours

after intravenous administration and 24 hours after intaperitoneal administration. This effect could be due to enhancement of peripheral glucose metabolism rather than an increase in insulin release (Gharaibeh ., et al, 1988).

Although the extract of *Cassia alata* has no effect on glucose levels in normoglycaemic animals, it reduced blood sugar value in streptozotocin-induced hyperglycaemic animals (Palanichamy ., *et al* 1988).

A single 0.5 ml oral dose of 40-80 % decoctions of *Trigonella foenum graceum* to normal as well as alloxanized mice was followed by hypoglycaemia developed over a 6-hour period. Reduction in blood glucose concentration was highly significant, and was maximum at 6 hours. It was dose-dependent. The hypoglycaemia caused by ethanol extract (200-400 mg/kg) in alloxanized mice was also dose-dependent and 200 mg/kg was comparable in effect to 200 mg/kg of Tolbutamide (Ajabnoor and Tilmisany 1988).

The powdered seeds of Acasia Arabica act by initiating the release of insulin from pancreatic beta cells of normal rabbits.Moreover, *Caralluma edulis* did not show any hypoglycaemic effect in normal as well as in diabetic rabbits (Wadood ., *et al*, 1989).

A hypoglycaemic effect due to an aqueous extract of *Tinospora crispa* stems was observed in moderately diabetic rats with concomitant improvement in insulinaemia. After a two- weeks treatment with the extract in drinking water, these rats also showed improvement in glucose tolerance. Moreover, acute intravenous treatment caused an increase in plasma insulin levels. The data support that. *Tinospora crispa* extract improves diabetic conditions by virtue of its action on the endocrine pancreas (Noor and Ashcroft 1989).

No seasonal variation in the hypoglycaemic activity of *Opuntia Streptacantha Lemaire* was detected, suggesting it can be used year round for treatment of Diabetes mellitus (Meckes ., *et al* 1989).

Momordica charantia Linn plant, *Coccinia indica* whit, Arm root and *Tricosanthes dioica Roxb*, significantly lowered blood sugar in fasted model and depressed the peak value in glucose loaded models (Chandrasekar ., *et al* 1989).

Single (100-400 mg/kg) oral doses of an alcoholic extract of *Zizyphus sativa* leaves to normal rats showed a dose-dependent statistically significant lowering of blood glucose 2, 4 and 6 hours later. The effect was most pronounced at 6 hours with glucose returning to control values at 24 hours. In alloxan-diabetic rats, no significant effect was observed (Anamd ., *et al* 1989).

A traditional treatment for diabetes mellitus, consisting of seven plants and a herbal mixture was given a 6.5 % by weight of the diet of streptozotocin diabetic mice for 9 days. The results suggest that bearberry (*Arctostaphylos uva – ursi*); golden seal (*Hydrastis canadensis*), mistletoe (*Viscum album*) and tarragon (*Artemisia dracuncuus*) may counter some of the symptoms of diabetes mellitus such as hyperplasia and polydipsia without affecting glycaemic control (Swanston and Flatt 1989).

The non- dialyzable portion of the water extract of the Oriental crude drug (Karokon), the roots of *Trichosanthes kirilowi*, was fond to reduce the plasma glucose level in mice. Five glycan termed as trichosans A, B, C, D, and E were isolated from this drug. The main glycan, trichosan, also exhibited activity in alloxan – induced hyperglycaemic mice (Hikino., *et al*1989).

Among twelve plants used for treatment of diabetes mellitus only Guayusa and mushroom retarded the development of hyperglycaemia in streptozotocin diabetic mice and reduced the hyperphagia, polidipsia,

body weight loss and glycated haemoglobin. Mushroom also countered the initial reduction in plasma insulin and the reduction in pancreatic insulin concentration and improved the hypoglycaemic effect of exogenous insulin. These studies suggest the presence of potentially useful anti-diabetic agents in guayusa and mushroom (Swanston and Flatt 1989).

Twenty eight medicinal plants used in the treatment of diabetes mellitus were studied for their anti-hyperglycaemic effect. Each plant was processed in the traditional way and intragastrically administered to temporarily hyperglycaemic rabbits. The results showed that 8 out of 28 studied plants significantly decreased the hyperglycaemic peak and / or the area under the glucose tolerance curve. The plants were:
Guazuma ulmifolia, Tournefoctia hirsutissima, Lepechinia caulescens, Rhizophora mangle, Musa sapientum, Trigonella foenum graceum, Tunera diffusa and *Euphorbia prostrate*. The results suggest the validity of their clinical use in diabetes control, after their toxicological investigation (Alarcon and Aguilaria ., *et al* 1998).

Eucalyptus globules (eucalyptus) are used as a traditional treatment for diabetes. In this study, incorporation of eucalyptus in the diet (62.5 g/kg) and drinking water (2.5 g/L) reduced the hyperglycaemia and associated weight loss of streptozotocin-treated mice. An aqueous extract of eucalyptus (0.5 g/L) enhanced 2- deoxy –glucose transport by 50 %, glucose oxidation by 60 % and incorporation of glucose into glycogen by 90 % in mouse abdominal muscle. Within 20 minutes aqueous extract of eucalyptus evoked a stepwise (70-160 %) enhancement of insulin secretion from the clonal pancreatic beta-cell line. These data indicate that Eucalyptus globulus represents an effective antihyperglycaemic dietary adjustment for the treatment of diabetes and a potential source for

discovery of new orally active agent(s) for future therapy (Gray and Flatt 1998).

The protective effect of *Anacardium occidentale* aqueous extract against streptozocin – induced diabetes was evaluated in rats. The rats were treated with 175 mg/kg of the extract per os, twice daily, beginning 2 days before streptozotocin (STZ) injection. A total of 3 days after STZ administration, there was a 48% increase in blood glucose level in pre-treated rats, compared with an increase in diabetic control rats treated with STZ alone. Furthermore, these pre-treated animals presented no glycosuria, a normal weight gain and a non significant increase in food and fluid intake at the end of the treatment compared with the normal control. Diabetic control animals showed a positive glycosuria, body weight loss, a real polyphagia and polydypsia. These results indicate the protective role of *Anacardium occidentale* extract against the diabetogenic action of STZ (Kamtchouing ., *et al* 1998).

The hypoglycaemic and anti – hyperglycaemic activities of methanol extract of *Mominda lucida Benth*. Leaves were studied in normal and streptozotocin (STZ) – diabetic rats .In normal rats, the extract demonstrated a significant ($P<0.05$) and dose–dependent hypoglycaemic activity within 4 hours after oral administration. After 12 hours, the plasma glucose level of rats administered 50, 100, 200 or 400 mg/kg of extract also decreased. In hyperglycaemic rats, the extract produced a significant ($P< 0.05$) anti – diabetic effect from day 3 after oral administration, with 400 mg/kg extract –treated animals having a plasma glucose level of 248.7 ± 5.3 mg/100ml compared with glibenclamide (10 mg/kg) with plasma level of 251.5 ± 5.8 mg/100 ml .These results suggest that the leaves of *Morinda lucida* have a strong

glucose lowering property when administered to STZ –treated rats (Olajide ., *et al* 1999).

This study was undertaken to investigate the effect of *Momordica cyambalaria* fruit powder on blood glucose and other biochemical parameters in alloxan – induced diabetic rats. The treatment was given for 15 days. After the treatment, a significant reduction was observed in fasting blood glucose levels in the treated diabetic rats, but no hypoglycaemic activity in the treated normal rats. *Momordica cyambalaria* treatment showed considerable lowering of serum cholesterol and triglycerides in treated diabetic group. There was a significant improvement in hepatic level after the treatment with the *M.cymbalaria*. These results suggest that the *Momordica cyambalaria* fruit powder possesses ant diabetic and hypolipidemic effects in alloxan – induced diabetic rats (Rao 1999).

The aqueous extract of the leaves of *Zizyphus mauritiana Lam* (Rhamnaceae) was studied for its antidiabetic effect. The extract was administered per os to Wistar rats made diabetic either temporarily by oral glucose tolerance test (first case) or definitely by subcutaneous injection of alloxan (second case). It was observed a striking decrease of the hyperglycaemic arrow ($p<0.05$) in the first case, with 300mg/kg administered 90 minutes before starting the test. In the second case , the results obtained with a dose of 300 mg/kg once or twice a day were identical as those with glibenclamide at 0.2 mg/kg per day .So , the antidiabetic activity of Zizyphus mauritana Lam. was experimentally born out but it has to be standardized for common use (Cisse ., *et al* 2000).

Gulnar farsi, male abortive flowers of *Punica granatum L.* was used for the treatment of diabetes mellitus in Unani medicine. Oral

administration of its aqueous – ethanol extract led to significant blood glucose lowering effect in normal, glucose-fed hyperglycaemic and alloxan-induced diabetic rats. This effect of the extract was maximum at 400 mg/kg body weight (Jafri ., et *al* 2000).

The hypoglycaemic effect of five Brazilian medicinal plants was studied on alloxan-induced diabetic rats. The extract of these plants were intragastrically administered to diabetic rats. The results showed that all plant studied (except one) significantly lowered the blood glucose. These results suggest that these four medical plants could be an adjuvant agent in the treatment of diabetes mellitus. The four plants were *Marrubium vulgare, Rubus imperialis* and *Wedelia poludosa* (Novaes ., *et al* 2001).

Hypoglycaemic activity was detected in dichloromethane methanol extract (111) of leaves and twigs of *Catharanthus roseus* (family Apocynaceae) a traditionally used medicinal plant using streptozotocin (STZ) induced diabetic rat model. Extract at dose 500 mg/kg given orally for 7 and 15 days showed 48.6% and 57.6% hypoglycaemic activity, respectively. Prior treatment at the same dose for 30 days provided complete protection against STZ challenge (75 mg /kg /I.P. X I). Enzymic activities of glycogen synthase, glucose-6-phosphate–dehydrogenase, succinate dehydrogenase and malate dehydrogenase were decreased in liver of diabetic animals and were significantly improved after treatment with extract at dose 500 mg/kg / p.o. for 7 days. Results indicate increased metabolization of glucose in treated rats. Increased levels of lipid peroxidation measured as 2-thiobarbituric acid reactive substances indicative of oxidative stress in diabetic rats were also normalized by treatment with the extract (Singh., *et al* 2001).

This study was executed to evaluate the anti-diabetic potency of a polyherbal formulation, and its influence on derangement in metabolism of glucose, cholesterol and sodium levels in normal and alloxan induced

diabetic rats. Serum glucose and cholesterol were found to be increased in diabetic animals. Serum sodium as well as urinary sodium, hepatic glycogen were found to be decreased in diabetic state .Treatment with the polyherbal formulation (1.0 ml/kg body weight) for 30 days in diabetic animals has shown decrease in serum glucose and serum cholesterol levels in comparison to control animals, whereas in normal treated animals, the formulation does not affect the serum glucose and serum cholesterol levels. Serum sodium and urinary sodium levels were increased in both diabetic treated and the control animals. Hepatic glycogen levels were increased in diabetic treated animals, but there was no change in the control treated animals (Annapurna ., *et al* 2001).

In the past 15 years, there have been controversial reports on the hypoglycaemic activity of Aloe species, probably due to difference in the parts of the plant used or to the model of diabetes chosen .In the study, separate experiments on three main groups of rats, namely non-diabetic type I (IDDM) and type II (NIDDM) diabetic rats were carried out. *Aloe Vera* leaf pulp and gel extracts were ineffective on lowering the blood sugar level of non diabetic rats. *Aloe vera* leaf pulp extract showed hypoglycaemic activity on IDDM and NIDDM rats, the effectiveness being enhanced for type II diabetes in comparison with glibenclamide. On the contrary, *Aloe vera* leaf gel extract showed hyperglycaemic activity on NIDDM rats .It may therefore be concluded that the pulps of *Aloe vera* leaves devoid of the gel could be used in treatment of non-insulin dependent diabetes mellitus (Okyar ., *et al* 2001).

The purpose of this study was to investigate the effects of daily oral feeding *Momordica charantia* (Mc) (200mg/kg) , *Eugenia jambolama* (200 mg/kg) , *Mucuna pruriens* (200 mg/kg) and *Tinospora cordifolia* extracts for 40 days on blood glucose concentrations and kidney functions in (STZ)-diabetic rats . Plasma glucose levels, body

weight, urine volume and urinary albumin levels were monitored on every 10 days over a 40- day's period, while plasma creatinine levels were assessed at the beginning and end of the experiment. Renal hypetrophy assessed the ratio between the kidney weight and total body weight. Plasma glucose concentrations in STZ–diabetic mice were reduced by the administration of extracts of *Momordica charantia* , *Tinospora cordifolia* , and *Mucuna pruriens* by 24.4 , 20.84 , 7.45 and 9.o7 % , respectively, ($P<0.005$) for *Momordica charantia*, *Eugenia jambolama* ,MP and (P 0.05) for *Tinospora cordifolia*) . Urine volume was significantly higher ($P<0.005$) in diabetic control and *Momordica charantia*, *Eugenia jambolama*, MP and TC treatment prevented polyuria ($P < 0.001$, 0.0001 0.01 and 0.001 respectively).After 10 days of STZ administration on urinary albumin level (UA) were over 6 folds higher in diabetic controls as compared to normal controls. Treatment with *Momordica charantia*, *Eugenia jambolama*, *Mucuna pruriens* and *Tinospora cordifolia* significantly prevented the rise in (AU) levels from day 0 to 40 in comparison to diabetic controls ($P<0.0001$, 0.0001, 0.005 respectively). Renal hypertrophy was significantly higher in diabetic controls as compared to non-diabetic controls. *Tinospora cordifolia* and MP failed to modify renal hypertrophy. Results indicate that these plant drugs should be studied further (Grover ., *et al* 2001).

The hypoglycemic activities of four water ethanol extracts (WEE) prepared from *Bidens pilosa L., Salvia officinalis L., Psacalium peltatum H.B.K. (Cass)* and *Turnera diffusa* Wild. Were investigated in healthy and alloxan-diabetic mice. The WEE of *S. officinalis* significantly reduced the fasting blood glucose of normal mice. The WEE of P. peltatum and *B. pilosa* also significantly diminished glycemia in healthy

mice. In mildly diabetic mice, the WEE of *Psacalium. peltatum* lowered the basal blood glucose level. The WEE of *Bidens pilosa* and *Salvia officinalis* also significantly diminished the hyperglycemia in mildly diabetic mice. The administration of these three extracts to animals with severe hyperglycemia did not cause a significant decrease. The WEE of *Turnera. diffusa* did not show any hypoglycemic activity. Thus, three of the WEE studied conserved the hypoglycemic activity originally detected in the traditional preparations of the studied antidiabetic plants. It appears that these extracts require the presence of insulin to show hypoglycemic activity (Alarcon and Aguilar 2002).

Gongronema latifolim is a rain forest plant, which has been traditionally used in the south eastern part of Nigeria for the management of diabetes. The effects of oral administration of aqueous and methanolic extracts of the leaves of *Gongronema. latifolim* for 2 weeks to STZ induced diabetic *rats were investigated. Results suggest that the extracts from Gongronem .latifolium* leaves could exert their anti-diabetic activities through their anti-oxidant properties (Ugochukwu ., *et al* 2002).

This study was undertaken to examine the hypoglycemic effect of aqueous extract of *Hypoxis hemerocallidea* (family: Hypoxidaceae) corm (locally known as "African Potato") in normal (normoglycemic) and in streptozotocin (STZ)-treated, diabetic rats., the results of this experimental animal study indicate that African potato possesses hypoglycemic activity; and thus lends credence to the suggested folkloric use of the herb in the control and/or management of adult-onset, type 2 diabetes mellitus in some communities of South Africa. (Mohamed ., *et al* 2003).

This study examined the hypoglycemic activity of *Cyclocarya paliurus* (Batal.) Iljinskaja in mice by oral glucose tolerance testing. The

blood glucose level was significantly lower in the *C. paliurus* extract treatment group than in the control group (Kurihara ., *et al* 2003).

To unravel the possible mechanism of glucose lowering activity, effects of ten different plant extracts in the regulation of serum cortisol and glucose concentrations were evaluated in male mice. While the extracts of *Inula racemosa, Boerhaavia diffusa* and *Ocimum sanctum* decreased the serum concentration of both cortisol and glucose, *Aegle marmelos, Azadirachta indica* and *Gymnema sylvestre* extracts could exhibit hypoglycaemic activity without altering the serum cortisol concentration. It appears that the hypoglycaemic effects of former three plant extracts are mediated through their cortisol inhibiting potency, whereas the mechanism for other plant extracts could be different. Lipid-peroxidation was not enhanced by any of the plant extracts (some were in fact, antiperoxidative in nature). As *Inula racemosa, Boerhaavia diffusa* and *Ocimum. sanctum* exhibited antiperoxidative, hypoglycaemic and cortisol lowering activities, it is suggested that these three plant extracts may potentially regulate corticosteroid induced diabetes mellitus (Gholap., *et al* 2004).

The hypoglycemic effect of aqueous extracts of *Carum carvi* and *Capparis spinosa L*. (CS) fruit were investigated in normal and streptozotocin (STZ) diabetic rats. After administration of the aqueous *Carum carvi* and CS extracts produced a significant decrease on blood glucose levels in STZ diabetic rats the blood glucose levels were nearly normalized 2 weeks after daily repeated oral administration of both aqueous *Carum carvi* and *Capparis spinosa L*. extracts. No highly significant changes on blood glucose levels were noticed in normal rats after both acute and chronic treatments with *Capparis spinosa L*. and *Carum carvi*. In addition, no changes were observed in basal plasma insulin concentrations after treatment with these plants in either normal or

STZ diabetic rats indicating that the underlying mechanism of this pharmacological activity seems to be independent of insulin secretion. We conclude that aqueous extracts of *Carum carvi* and *Capparis spinosa L.* exhibit a potent anti-hyperglycaemic activity in STZ rats without affecting basal plasma insulin concentrations (Eddouks ., *et al* 2004).

Cissus sicyoides (Vitaceae) is a medicinal plant popularly known in Brazil as "cipo-puca, anil-trepador, cortina, and insulina". The plant is used in treatment of diabetes. In the present work. The hypoglycemic and anti-lipedemic effects of the aqueous extract prepared from fresh leaves of the plant were studied in the model of alloxan-induced diabetes in rats. The Results showed that the daily treatment of diabetic rats with aqueous extract prepared from fresh leaves of *Cissus sicyoides* for 7 days significantly decreased blood glucose levels, a significant decrease was observed in plasma triglyceride levels (Viana ., *et al* 2004).

The effect of an aqueous extract of *Origanum vulgare* (OV) leaves on blood glucose levels was investigated in normal and streptozotocin (STZ) diabetic rats. In normal rats, the blood glucose levels were slightly decreased 6 hrs after a single oral administration of the aqueous extract produced a significant decrease on blood glucose levels in STZ diabetic rats ($P<0.001$) (Lemhadri ., *et al* 2004).

The hypoglycemic effect of the aqueous extracts of *Fraxinus excelsio*r seeds and *Silybum marianum* aerial part was investigated in normal and streptozotocin (STZ) diabetic rats. After a single dose or 15 daily doses, oral administration of the aqueous extracts (20 mg/kg) produced a significant decrease of blood glucose levels in both normal and STZ diabetic rats ($P < 0.001$). From the first week, the body weight was increased in normal rats ($P<0.05$) and decreased in STZ rats ($P<0.01$) after FE administration. In addition, no changes were observed in basal plasma insulin concentrations after both FE and SM treatments in either

normal and STZ diabetic rats indicating that these plants exert their pharmacological activity without affecting insulin secretion. It is concluded that the aqueous extracts of *Fraxinus excelsio*r and *Silybum marianum* exhibit potent hypoglycemic and anti-hyperglycemic activities in normal and STZ rats, respectively, without affecting basal plasma insulin concentrations (Maghrani ., *et al* 2004).

The fruit of *Tetrapleura tetraptera* (Taub) [Fabaceae] is frequently used in Tropical African traditional medicine for the management and/or control of diabetes mellitus. The present study was undertaken to examine the hypoglycemic effects of *Tetrapleura tetraptera* (Taub) fruit aqueous extract in rats. Streptozotocin (STZ)-induced diabetes mellitus was used as an experimental test model of diabetes. The plant extract produced dose-dependent, significant reductions ($P < 0.05 - 0.001$) in the blood glucose concentrations of both fasted normal and fasted diabetic rats. The results of this experimental animal study indicates that *Tetrapleura tetraptera* fruit aqueous extract possesses anti-inflammatory and hypoglycemic properties. These findings lend pharmacological credence to the suggested folkloric uses of the plant's fruit in the management and/or control of arthritis and other inflammatory conditions, as well as in adult-onset, type-2 diabetes mellitus in some Yoruba-speaking communities of South-Western Nigeria (Ojewole., *et al* 2004).

This study indicates that the crude methanolic extract of *Loranthus micranthus* (Linn.) exhibited statistically significant hypoglycemic and anti-hyperglycemic activities in normoglycemic and alloxan-induced diabetic albino rats.

The methanolic extract of African mistletoe was found to be a good candidate for alternative and/or complimentary medicine in the management of diabetes mellitus. The leaves of the Eastern Nigerian

species of the African mistletoe harvested from *Kola acuminata, Azadirchta indica* and *Baphia nitda* host trees exhibited comparatively better anti-hyperglycemic activities among the host trees studied(Osadebe ., *et al* 2004).

In order to appraise some of the ethno medical uses of Sclerocarya (A. Rich.), this study was undertaken to investigate the analgesic, anti-diabetic properties of the plant's stem-bark aqueous extract in experimental models of diabetes mellitus. The analgesic effect of Sclerocarya birrea stem-bark aqueous extract was evaluated in mice while anti-diabetic effects were investigated in rats. The results of this experimental animal study indicate that *Sclerocarya birrea* stem-bark aqueous extract possesses analgesic and hypoglycemic properties (Ojewole 2004).

In this study, the preventative anti-diabetic and anti-obese effects of wild Ginseng (a shade-loving herb) ethanol extract (WGEE) were investigated. In the preventive experiment, WGEE co-administered with a high fat diet significantly inhibited body weight gain, fasting blood glucose, triglyceride, and free fatty acid levels in a dose dependent manner. WGEE-treated mice at doses of 250 and 500 mg/kg improved the insulin resistance index by 55% and 61% compared to the high fat diet control, respectively. Diameters of white and brown adipocytes were also decreased by 62% and 46% in the WG500-treated group compared to those in HFD fed control mice. Taken together, wild Ginseng ethanol extract (WGEE) has potential as a preventive agent for type 2 diabetes mellitus (and possibly obesity) and deserves clinical trial in the near future (Yun., *et al* 2004).

The hypoglycemic effect of *Ganoderma lucidum* polysaccharides (Gl-PS) was investigated in the normal fasted mice. It is found that Gl-PS possesses the hypoglycemic effect on normal mice through its insulin-

releasing activity due to a facilitation of Ca^{2+} inflow to the pancreatic beta cells (Zhang., *et al* 2004).

Cogniauxia podoleana Baillon leaves are used in Congolese traditional medicine for the treatment of diabetes mellitus. Based on an increasing number of reports on blood glucose level reduction associated with some saponins and flavonoids isolated from medicinal plants, the hypoglycemic and antihyperglycemic effects of flavonoid and saponin fractions were investigated (Diatewa ., *et al* 2004).

Ferula persica, Paronychia argentea, and *Pistacia atlantica* are three of the plants widely recommended by the herbalists and used for their hypoglycemic activity in Jordan. Aqueous extracts of these plants were tested in vitro for their hypoglycemic activity in normoglycemic and streptozocin-induced hyperglycemic rats. Although the three plants were advocated for their hypoglycemic effects in Jordanian traditional herbal medicine; none of them showed significant hypoglycemic activity compared to the untreated animals (Hamdan., *et al* 2004).

Oral administration of the aqueous extract of *Scoparia dulcis* plant extract to diabetic rats led to decreased levels of blood glucose and plasma glycoproteins. The levels of plasma insulin and tissue sialic acid were increased whereas the levels of tissue hexose hexokinase and fructose were near normal. This study indicates that S.dulcis possesses a significant beneficial effect on glycoproteins in addition to its antidiabetic effect (Latha and Pari 2005).

1-2-3-8- Hypoglyacemic Medicinal Study Plants

1-2-3-8-1- *Cicer arientinum*

1-2-3-8-1-1- Morphology:

Erect shrubby quick-growing annual about 50 cm high,

Photo (1-1): *Cicer arientinum*

General taxonomy:

Arabic Name:الحمص كبكبي

English Name: chick peagarbanza

Latin name: *Cicer arientinumL*

Family: Fabaceae

Habitat: Northern Sudan

1-2-3-8-1-2- Chemical constituent:

Volatile oil, amino acids and starch.

1-2-3-8-1-3- Pharmacology:

The active ingredient**,** Daphnethin, has an antibacterial activity on both gram-positive and gram – negative bacteria. Genistein has an antifungal activity. The seeds are stimulant and tonic, used in skin diseases and for nourshing hair and face (Harborne 1999).

1-2-3-8-1-4- Toxicology:

Cicer is edible and no adverse effects are reported from this plant (Harborne 1999)

1-2-3-8-1-5- Folkloric Use:

1-2-3-8-1-6- Treatment of obesity and diabetes mellitus(Elghazali *et al,* 1994).

1-2-3-8-2- *Cinnamomum verum*

1-2-3-8-2-1- Morphology:

It is a moderate sized tree. The bark is smooth, light pinkish brown and thin, with a strong, pleasant small and spicy, burning taste.

Photo (1-2): ***Cinnamomum verum***

1-2-3-8-2- 2- General taxonomy:

Arabic Name: القرفة

English Name: Cinnamon, Ceylon cinnamon

Latin name: *Cinnamomum verum*

Family: Lauraceae

Habit**at:** India

1-2-3-8-2-3- Chemical constituent:

Volatile oil, cinnamic aldehyde and terpenoides.

1-2-3-8-2-4- Pharmacology:

The active ingredient on cinnamon turned to be water-soluble poly phenol compound called MHCP. This compound mimics insulin, activates its receptor, and works synergistically with insulin in cells. Cinnamon can improve glucose and cholesterol (Alam Khan 2003)

1-2-3-8-2-5- Folkloric Use:

Renal diseases, **diabetes mellitus**, productive cough, CNS stimulant, memory activator and menstural cycle stimulant. (Elghazali., *et al* 1994). The essential oil in Cinnamon has demonstrated strong antibacterial and antifungal properties (Bruneton 1995).Cinnamon bark has also shown strong lipolytic activity, hydrolysig fats (Leung and Foster 1996).

1-2-3-8-3 -*Citrus aurantifolin*

1-2-3-8-3-1- Morphology:

A spine scent tree up to 6 m in height.

Photo (2-3): *Citrus aurantifolin*

1-2-3-8-3-2- General taxonomy:

Arabic Name:الليمون

English Name: Lime

Latin name: *Citrus aurantifolin*

Family: Rutaceae

Habitat: Various areas

1-2-3-8-3- 3- Chemical constituent:

Volatile oils, terpeins, hesperidin and vitamin B, citric acid, vitamin C, potassium and calcium citrate and flavinoides.

1-2-3-8-3- 4- Folkloric Use:

The stem is used as an antiseptic for mouth, the juice is used for treatment of rhinitis and cold and can be added to coffee and tea for

treatment of diarrhoea, the boiled leaves can be used for treatment of **diabetes mellitus.** (Elghazali., *et al,1994.*

1-6- Objectives:

1-This study is carried out to confirm the folkloric use of *Cicer arientinum, Cinnamomum verum* and *Citrus aurantifolin* in treatment of diabetes mellitus.

2-To determine the effectiveness of these plants as hypoglycaemic agents compared with known reference drugs as Glibenclamide for non insulin-dependent diabetes mellitus (NIDDM) and Insulin for Insulin –dependent diabetes mellitus (IDDM).

3-To evaluate the effects of these plants on plasma total cholesterol and plasma triglyceride levels in (NIDDM) and on (IDDM).

4-To evaluate the toxicity of these plants in terms of their effects on haematological parameters, biochemical parameters (Liver and Kidney function tests) and histopathological changes.

5-To determine the pharmacological effects of these plants on isolated body tissues.

6-To phytochemically screen these plants for their chemical constituents.

7-To formulate these plants in suitable pharmaceutical forms to be used by diabetic patients as oral hypoglycaemic agents.

Chapter Two

2- Materials and Methods

2-1 Study area:

The hypoglycaemic effect of the study plants in type II diabetic rats was studied at the research laboratory – **Faculty of pharmacy** – University of Khartoum, while that for type I, together with the toxicological study, were performed at the **Medicinal and Aromatic Plants Research Institute** (MAPRI) – National Centre for Researches (Khartoum).The biochemical analysis was carried out at The Diagnostic Research Unit (**Khartoum Hospital**).The histopathological investigation was conducted at the Histopathology laboratory – **Faculty of Veterinary Medicine** – University of Khartoum.

2-2 The study plants:

The experimental plants used in this study were the seeds of *Cicer arientinum*, the bark of *Cinnamomum verum* and the leaves of *Citrus aurantifolin*.

2-2-1 Source of the study plants:

C.arientinum and *C.verum* were obtained from Omdurman General Market while *C.aurantifolin* was obtained from a home-garden in Omdurman.

2-3. The study animals:

Photo (2-1): Group of Wistar Albino Rats.

350 adult male Wistar albino rats, weighing 100-300 grams were used throughout the course of this study. 140 rats represented type II, 140 rats were used for type I and 70 rats were used for the toxicological study. All rats were obtained from the Faculty of Pharmacy; University of Khartoum. The diet given to the rats consisted of (meat + flour + milk + salt + oil) besides water ad libidum.

2-3-1 Study groups:

2-3-1-1 Controls:

30 rats were distributed among 3 control groups of 10 rats each. One group represented the control for type I, another for type II and a third one for the toxicological study.

2-3-1-2 Standards:

20 rats were distributed into two standard groups. The standard reference drug for type I, was Insulin and that for type II was Glibenclamide.

2-3-1-3 Samples:

2-3-1-3-1 Sample groups for Types II and I diabetes mellitus:

In type I as well as type II, each of the plants was studied in terms of two extracts (aqueous and methanolic) and each extract was studied in terms of two doses (400 and 200 mg/kg). Thus 40 rats were used by each plant for each type; consequently the total number of rats used as samples for investigation of the ant diabetic effects of these plants was 240 rats.

2-3-1-3-2 Samples for the toxicological study:

This part dealt with the two doses of the methanolc extracts of the 3 study plants. Thus **60** rats were utilized.

2-4 Chemicals, reagents and Equipments:

Table (2-1) **Materials** - Chemicals and reagents

Chemicals	
2% hydrochloric acid	BDH chemical LTD; Poole, England
2% potassium Ferro cyanide	BDH chemical LTD; Poole, England
Acetic anhydride	E- Merch, Darmstadt A rt.42
Benzene (Benzol)	E merch, Darmstadt, Art.1783.
Chloroform AR	Analytical Rasyan
Concentrated sulphuric acid.	Analytical Rasyan
Cristaseal	Hawksley, England
DPX-mountant	Avishkar, India
Eosin	BDH chemical LTD; Poole, England
Ethanol	BDH chemical LTD; Poole, England
Ethanol 80 %	Riedgl-DE. Haen AG SEELZE-Hannover
Ethyl acetate.	Analytical Rasyan
Ethylene diamine tetra acetic acid (EDTA)	Analytical Rasyan
Formal Saline (Haymes Solution).	Analytical Rasyan
Formaldehyde	Fisher Scientific Company; New Jersey
Glibenclamide	Sigma, mixed anomer lot 74 F
Glucose 50%	Analar (AR)
Haematoxylin	Sid-fine chem., India
Halothane	BP ICI India Limited, Ennor, Chennai-600 057.
Hydrochloric acid HCl 2N	Analytical Rasyan

Hydrogen peroxide 3%	B.P. Bell Sons & Co. LTD; South Port PRQ 9AL, England.
Insulin (Soluble)	
Methanol	Analytical Rasyan.
n-hexane	LOBA CHEMIE PVT. LTD.Mumbai-400002 Ind
Paraffin wax	Burgoyne-India, Mumbai
Petroleum ether	E.Merck (India) Limited, Mumbai-400 018.
Plant Extract (Aqueous and alcoholic)	
Potassium hydroxide 0.5N	Scharlau Chemie S.A. ITD England.
Sodium picrate paper (Picric acid)	BDH Laboratory Supplies Poole, BH 15 ITD, England.
Sodium sulphate anhydrous	Chadweil Health ESSEX-England.
Standard haemoglobin	Cromatest-ISO 9001
Streptozotocin	Sigma S 0130.
Xylene	Carlo erba, Milano
Kits	
Alkaline phosphatase kits	Plasmatec Laboratory products LTD. Bridport, Dorset DT6 5BU. U.K.)
Cholesterol kits	Plasmatec Laboratory products LTD. Bridport, Dorset DT6 5BU. U.K.
Creatinine kits	Plasmatec Laboratory products LTD. Bridport, Dorset DT6 5BU. U.K.)
Glucose kits	Plasmatec Laboratory products LTD. Bridport, Dorset DT6 5BU. U.K.

GOT kits	Plasmatec Laboratory products LTD. Bridport, Dorset DT6 5BU. U.K.)
GPT kits	Plasmatec Laboratory products LTD. Bridport, Dorset DT6 5BU. U.K.)
Triglyceride kits	Plasmatec Laboratory products LTD. Bridport, Dorset DT6 5BU. U.K.)
Urea kits	Plasmatec Laboratory products LTD. Bridport, Dorset DT6 5BU. U.K.)
Reagents	
Diluting solution for total RBCs	3.8% w/v tri sodium citrate plus 5 ml 10% formaldehyde were added to 495 ml of Formal Citrate.
Diluting solution for total WBCs	1.5ml glacial acetic acid and 1ml 1% of an aqueous solution of gention violet were added to 98 ml of D.W.
Drabkin`s solution for haemoglobin	0.2g potassium ferric cyanide &1g sodium bicarbonate were dissolved in 1 litre of D.W.
Mayer's reagent	(1) 1.36 gm of $Hgcl_2$ were dissolved in 60 ml distilled water (2) 5 gm of potassium iodide were dissolved in 10 ml water; Solution (1) & (2) were mixed and distilled water was added up to 100 ml).
Vasler`s reagent	10 gm of potassium iodide was dissolved in 100 ml of water, and

	then mercuric iodide was added until saturation. Excess mercuric iodide was removed.

(Table 2-2) Equipments

Equipment	**Source**
Centrifuge	MSE
Centrifuge tubes	MSE
Centrifuge tubes / Centrifuge	Hettich EBA 35 Werk-Nr, Baujahr
Freeze drier	ALPHA-1-12 W.Germany.
Frosted one end side slide	Schniewindt
Haematokrit capillary tubes Microhaematokrit Centrifuge	SUPE-PIOR-Germany. Code No.4361 Hawksley & Sons Ltd., England.
Heparinized capillary tubes	Supe-Rior-Germany, Code NO.4361
Hotplate	Herraeus (dehydrater)
Light microscope	Olympus Optical.
Measuring cylinders (50ML)	Volac
Microhaematokrit reader	Hawksley, England.
Micropipettes 5-50 ul	Volac
Microscopic cover glass 24 x 50 mm	Schniewindt
Microtome	Medax
Neubauer haemocytometer	ENM-England.
Oven used to melt wax	Schniewindt
Oven used to melt wax	Schniewindt
Pasteur pipettes	Hettich EBA 35 Werk-Nr, Baujahr
Photographic microscope	Leitz-Dialux 20
Rotatavapor	BUCH1 011, Switzerland.
Rough balance	Sartorius 2351, Germany.

Naso -gastric feeding tubes	Ch/FR (8) Kawamoto Corporation Osaka Japan.
Sample containers	AFMA – DISPO.
SB water bath	Fisher.
Sensitive balance	HF-300G A&D Company, Limited.
Soxhlet apparatus	Quick Fit, EX 5/83.England.
Spectrophotometer	Jenway 6305 UV/Vis., Jenway LTD, UK
Spectrophotometer	Jenway 6305 UV/VIS
Syringe s 5 ml/c	Chunjee Medical Industry CO. Koria.
Syringes 1 ml/cc	NeojectNeomedic LTD-Middlesex U.K
Vacuum embedding oven	Schniewindt
Water bath	Fisher
Water bath.	BUCH1 416 Switzerland.
Yellow tips	Volac

2-5 Methods

2-5-1 Plants:-

2-5-1-1 Preparation of the aqueous extract:

100 grams of the seeds of *Cicer arientinum*, 100 grams of the bark of *Cinnamomum verum* and 50 grams of the leaves of *Citrus aurantifolin* were weighed and each was just covered with boiling distilled water in a separate flask. They were incubated in a water bath at 60°c for four hours after which they were filterted. The filtrates were freez- dried. The dry powders were sealed in separate containers till use.

2-5-1-2 Preparation of the alcoholic extract:

60 grams of each plant were weighed and packed in soxhlet apparatus.500 ml of petroleum ether was used as a solvent for each to separate lipids and terpenoids. The samples were then unpacked and left to dry. The same samples were repacked in soxhlet and Chloroform was used as a solvent for the same purpose (separate lipids and terpenoids). The samples were again unpacked, dried and repacked again, this time with alcohol as a solvent to get the polar constituents of the plants. The extracts were evaporated till dryness using a rotatory evaporator, (Harborne 1999).

2-5-1-3 Phytochemical screening:-

A general screening was performed to identify the chemical constituents of the plants using the following methods.

2-5-1-3-1 Preparation of the extract for the phytochemical screening:

10 grams of the powdered tested part of the plants were refluxed with 100 ml of 80% ethanol in a round bottle flask for 4 hours. The cool solution was filtered and 80% ethanol was re-added to complete the filtrate to 100 ml. The prepared extract (PE) was used for the following standard tests:

2-5-1-3-2 Test for unsaturated sterols and triterpenses:-

10 ml of the (PE) was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. 5 ml portion of the chloroform solution was mixed with 0.5 ml of acetic anhydride followed by 2 drops of concentrated sulphuric acid. The gradual appearance of green , blue and pink to purple color was taken as an evidence for the presence of sterols (green to blue) and as triterpenses (pink to purple) in the sample.

2-5-1-3-3 Test for alkaloids:

7.5 ml of the (PE) was evaporated to dryness on a water bath. 5 ml of 2N HCL was added and stirred while heating on the water bath for 10 minutes, cooled, filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent was added while to the other tube few drops of valser`s reagent was added. A slight turbidity as heavy precipitate in either of the two test tubes was taken as a presumptive evidence for the presence of alkaloids.

2-5-1-3-4 Test for flavonoids:

17.5 ml of (PE) was evaporated to dryness in a water bath, cooled and the residue was defatted by several extractions with petroleum ether and the defatted residue was dissolved in 30 ml of 80% ethanol and filtered. To 3 ml of the filtrate in a test tube, 1 ml of 1 % potassium hydroxide solution was added. A dark yellow colour indicated the presence of flavonoid compounds.

2-5-1-3-5 Test for tannins:-

For this test 7 ml of the (PE) was evaporated to dryness on water bath. The residue was extracted several times with n – hexane and filtered. The insoluble residue was stirred with 10 ml of hot saline solution .The mixture was cooled, filtered and the volume of the filtrate was adjusted to 10 ml with more saline solution. 5 ml of this solution was treated with few drops of gelatin salt reagent. Formation of immediate precipitate was taken as evidence for the presence of tannin in the plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green colour was taken as an evidence for the presence of tannins.

2-5-1-3-6 Test for saponins:

I g of the original dried powder plant material was placed in a clean test tube. 10 ml of distilled water was added and the tube was stoppered

and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of (honeycombs whose persistent appearance for at least an hour), was taken as an evidence for the presence of saponins.

2-5-1-3-7 Test for cyanogenic glycosides:

3 grams of the powdered plant sample was placed in an Erlenmeyer flask and sufficient water was added to moisten the sample, followed by 1 ml of chloroform (to enhance every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split cork which was used to stopper the flask .A change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycoside .

2-5-1-3-8 Test for Anthraquinone glycoside:-

1 gram of the powdered plant sample was boiled with 10 ml of 0.5 N KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene and 5ml of the benzene solution was shaken with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinone was indicated by pink and red colour.

2-5-1-3-9 Test for coumarins:

1 gram of the original powdered plant sample was boiled with 20 ml distilled water in a test tube. A filter paper was attached to the test tube in order to be saturated with the vapor after adding to it 0.5N KOH, then the presence of cummarins was indicated if the spot was found to adsorb the UV light.

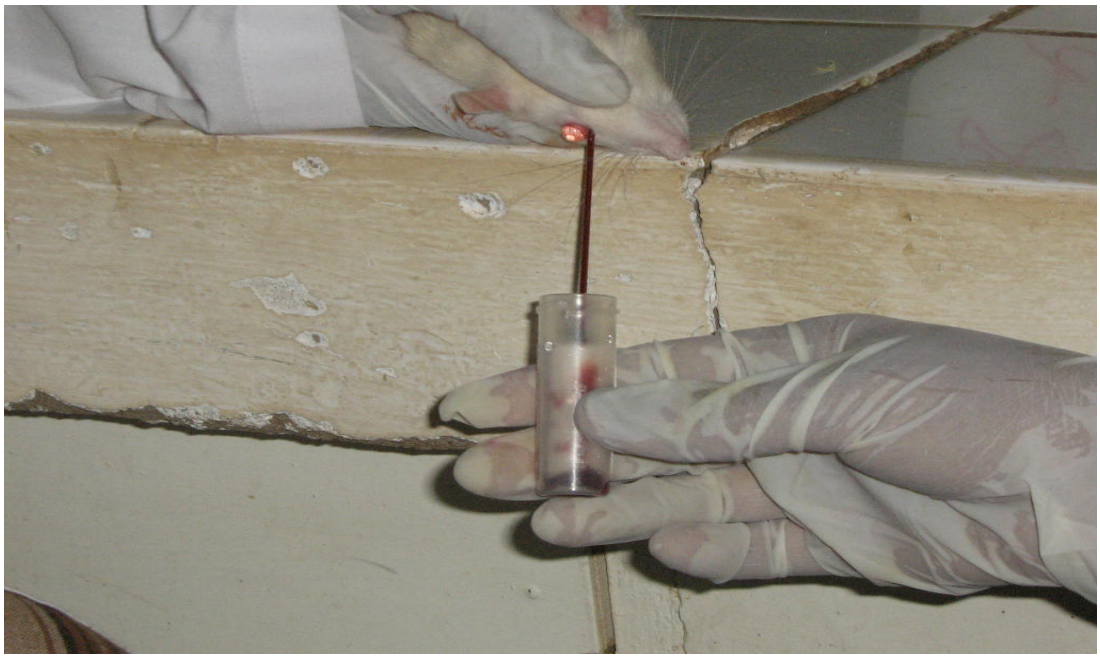
2-5-2 Animals

Experiments performed in animals were three, two of which dealt with studying the hypoglycaemic effects of the three plants (*C.arientinum, C.verum* and *C.aurantifolin*) and one dealt with studying the toxicity of the target plants.

2-5-2-1 Sample collection:

Blood samples were collected from the retro-orbital plexus of rats using heparinized capillary tubes (Khanna et al., 1992). This procedure was performed after anaesthetizing the rats through inhalation of halothane soaked in a piece of cotton bedded in a glass jar.

Photo -2: **Collection of blood samples from the Retro-Orbital Plexus of the rat's eye.**



2-5-2-2 Preparation of plasma:

Blood samples for measurement of glucose were collected into fluoride oxalate sample containers to prevent glycolysis, while blood samples for the other parameters (cholesterol, triglycerides, urea, creatinine, Na, K, GOT, GPT and ALP) were collected in heparinized sample containers and after mild shaking were centrifuged at 3000

revolutions/minute (rpm) for 15 minutes. The fluid part (plasma) was separated from the cellular part using a dropper and the plasma was placed in a new plane sample container labeleds according to the study group, rat number, time and date of collection.

2-5-2-3 Experimental design for the hypoglycaemic effect :

The experimental design of this study was based on the known glucose tolerance test (GTT), , except that intraperitoneal route was used rather than the oral route, for very rapid intery of glucose into the blood circulation through the portal vein. This group represent a reversible type II (NIDDM) induced by an intra-peritoneal loading dose of 50% glucose at a dose of2g/kg body weight.

2-5-2-3-1 Experimental procedure for the hypoglycaemic effect of the study plants in type II :

Animals were allowed to feed and drink ad libidum three days before the experiment. Then the target animals whether controls , standards or tests , were allowed to fast for eighteen hours by removal of all food materials except water. They were weighed and marked with serial numbers and the doses were calculated according to individual body weights. After the eighteen hours fast, blood samples were obtained from the retro orbital plexus (Khanna *et al*., 1992), using heparinized capillary tubes. Samples were collected at 0, 1, 2 and 4 hours post dosing. The 0 time represented the fasting blood samples while the 2 hours samples represented the post prandial sample. After collection of blood a calculated loading dose of glucose 2g /kg body weight of 50% glucose (konuklugil ., *et al* 1997) was given intraperitoneally (I/P) to each rat by means of (1ml Syringe). Simultaneously a dose of water 10 ml /kg body weight. (Aguilar., *et al* 1993) was administered orally to control rats by means of a gastric tube. The time after administration of doses was noted. Blood samples for the 1, 2 and 4 hours were collected in the

same way. All samples were centrifuged at 3000 revolutions per minute (3000 r.p.m) for 10 minutes after which plasma was separated from the cellular part of blood by means of Pasteur pipettes .The glucose concentration was immediately determined by means of plasmatic kits. The remaining plasma was kept freezed (till use) for the biochemical analysis of cholesterol and triglycerides. The some steps were followed for the standards and sample groups except that for the standard glibenclamide, 10 mg / kg .body weight at a concentration of 2 mg /ml of water given orally instead of water. The sub groups of samples received two doses (200 and 400mg/kg.b.w) of the aqueous and alcoholic extracts of the study plants orally.

2-5-2-3-2 Experimental procedure for the hypoglycaemic effect of the study plants in type I diabetic rats:

The same steps were also followed except that this time type I diabetes mellitus was induced in rats by intraperitoneal injection of streptozotocin (STZ) at a dose of (60 mg / kg b wt), dissolved in citrate buffer at a concentration of 20mg /1ml to provide a pH of 4.5 (Rakieten *et al* 1963). 48 hours latter, blood glucose was determined and those who became diabetic were chosen for the study.

Soluble insulin at a dose of 3U/kg (diluted 100 times) was used as standard for type I diabetes mellitus (instead of glibenclamide). Samples were collected at 0, 4, 8 and 12 hours (Suba *et al* 2004).

2-5-2-4 Experimental design for the toxicological study:

A toxicological study was performed by studying the effects of the two doses of the alcoholic extracts on the blood haemogram as well as on the liver and kidney functions. Furthermore half the lethal dose (LD_{50}) was determined.

2-5-2-4-1 Experimental procedure for the toxicological study:

A sub-chronic toxicological study was performed for the three plants. The control group was given a dose of water from day (0) until day (21), while the tested groups were given 200 and 400 mg/kg /b.w. of the alcoholic extracts of *C.arientinum*, *C.verum* and *C.aurantifolin* respectively. Blood samples were collected at day (0) and day (21) for determination of blood haemogram (T.WBCs , T.RBCs &, Hb) liver function tests (Alanine amino transferase (ALT=GOT), Aspartate aminotransferase (AST=GPT) and Alkaline phosphatase (ALP)) and kidney function tests (urea, creatinine Na and K).The experimental rats were dissected at day (21)and their organs were cut in appropriate sizes and fixed in 10%formalin and then were used for histopathological investigation.

Half the lethal dose (LD_{50}) was determined for the study plants by oral administering of different doses of the extracts to experimental rats until half the number of rats is dead.

2-5-2-5 Experimental design for the Pharmacological study:

A pharmacological screening was conducted in the rabbit intestine for determination of the effective dose and investigation of the mechanism of action of the study plants according to the method described by .Kitchen, 1984.

2-2-2-6- Experimental procedure for histopathological study:

The specimens were collected immediately after slaughtering and fixed in 10% formaldehyde, embedded in paraffin wax, sectioned at 5µm and stained. The sections were examined and photographed by a phase contrast microscope. The histopathological processing included fixation, dehydration, clearing, wax impregnation, section cutting, staining, examination and photographing by a phase contrast microscope, according to (Roy M. 1973)**.**

2-2-2-6-1- The staining process:

The staining process was performed using Haematoxylin and eosin according to Bancroft and Gumble, 2002. Light microscopic examinations were carried out at magnifications of 4 xs, 10 xs and 40 xs. According to Ustundag *et al*., 2000.

2-2-2-7- Haematological Investigations:

Red Blood Cells count (RBCs count), white blood cells count (WBCs count), and Haemoglobin concentration [Hb], were measured.
Blood samples were collected into dry clean bottles; the anticoagulant was ethylene diamine tetra acetic acid (EDTA).

2-2-2-7-1- Red blood cells (RBC) count:

Total erythrocytes were counted according to the method described by Dacie 1998.

2-2-2-7-2- Total White blood cells (TWBC) count:

White cells were counted according to Lewis et al., 2001.

2-2-2-7-3- Haemoglobin concentration [Hb]:

The concentration of haemoglobin was measured by the cyanomethaemoglobin technique according to the method described by Dacie 1998 .

2-2-2-8- Plasma Biochemical Analysis:

2-2-2-8-1- Glucose Oxidase Method:

the glucose concentration in blood, was determined using kits according to the method described by Trinder 1969.

2-2-2-8-2- Cholesterol Enzymatic Colorimetric Test: CHOD-PAP:

It is an enzymatic method; for measuring total cholesterol concentration in the serum or heparinized plasma described by Richmond (1974), and Zoppi and Fellini (1976) using kit.

2-2-2-8-3- Triglyceride Enzymatic Test:

The triglycerides are enzymatically hydrolyzed to glycerol as reported by Richmond (1974), and Zoppi and Fellini (1976)

2-2-2-8-4- Glutamate Oxaloacetate Transaminase (GOT):

=Aspartate amino transferase (AST):

Plasma GOT activity was determined by colorimetric method, as described by Cheesbrough 1987.

2-2-2-8-5- Glutamate Pyruvate Transaminase (GPT):

= Alanine amino transferase (ALT):

Plasma GPT activity was determined by colorimetric method, as described Karmen 1955 **,** using kit,

2-2-2-8-6- Alkaline Phosphate (ALP):

Alkaline phosphate activity was measured calorimetrically by a method similar to the procedure described by Chemie (1972).

2-2-2-8-7- Creatinine Determination:

Creatinine was determined by the method described by Slot (1965).

2-2-2-8-8- Urea Determination:

Plasma urea level was measured by the enzymatic method described by Marsh and Miller (1965), using kit.

2-2-2-8-9- Sodium Determination:

Sodium was determined by the standard procedure of flame emission spectrophotometry at 589nm.

2-2-2-8-10- Potassium Determination:

Potassium was determined by the standard procedure of flame emission spectrophotometry at 768nm.

2-2-2-9-Statistical Analysis:

Mean values in serum parameters were compared using the the SPSS - student's t-test. Data were expressed in mean ± standard error of mean (Mendenhall 1971).

2-2-2-10- Conversion of Animal Doses to Human Equivalent Doses (HED):

The human equivalent dose was calculated for each plant extract according to the guidance prepared by the Office of New Drugs in the Centre for Drug Evaluation and Research (CDER) in cooperation with the Centre for Biologics Evaluation and Research (CBER) at the United States - Food and drug Administration, December 2002.

2-2-2-10-1-Preparation of Antidiabetic Capsules of *Cicer arientinum*:

The (HED) for *C. arientinum* was found to be 1gram, thus o.5 grams of the completely dried alcoholic powdered extract was packed in (zero – size) gelatin capsules using the manual capsule machine. 2 capsules are recommended to be taken by the diabetic patient per day, to correspond the HED.

2-2-2-10-2-Preparation of Antidiabetic Capsules of *Cinnanomum verum*:

The (HED) for *C. verum* was found to be 1gram, thus o.5 grams of the completely dried alcoholic powdered extract was packed in (zero – size) gelatin capsules using the manual capsule machine. 2 capsules are recommended to be taken by the diabetic patient per day, to correspond the HED.

2-2-2-10-3-Preparation of Antidiabetic Capsules of *Citrus aurantifolin*:

The (HED) for *C. aurantifolin* was found to be 2gram, thus o.5 grams of the completely dried alcoholic powdered extract was packed in (zero – size) gelatin capsules using the manual capsule machine. 4 capsules are recommended to be taken by the diabetic patient per day, to correspond the HED.

Chapter Three

3-Results

This research studied the hypoglycaemic activity, pharmacology and toxicology of the aqueous and alcoholic extracts of *Cicer arientinum*, *Cinnamomum verum* and *Citrus aurantifolin* in hyperglycaemic rats (type II) and in sterptozotocin-induced diabetic rats (type I).

3-1-Yield of extracts

The alcoholic extract of *C.arientinum* was watery and yellow, *C.verum* was sticky and brown and that of *C. aurantifolin* was sticky and green.

All aqueous extracts were in powder-form (freeze- dried).The powder of *C. arientinum* revealed a yellow colour, *C.verum* a brown colour and *C. aurantifolin* a green colour.The yield percentage was calculated for the for the three study plants. The aqueous extract of *C.arientinum* yield was o.7% and its alcoholic yield was 1.4%. The aqueous extract of *C.verum* yield was 1.0% while its methanolic extract yielded 2.6%. The aqueous extract of *C.aurantifolin* yield was 5.3% and its alcoholic extract yield was 10.7%., as demonstrated in table (3-1).

3-2-Phytochemical Screening

General phytochemical screening was performed for *C.arientinum, C.verum* and *C. aurantifolin* to identify their chemical constituents.**.** Alkaloids and saponins were common constituents of the three study plants. Besides that *C.arientinum* revealed presence of sterols, cyanigenic glycosides and coumarins**.** *C.verum* revealed presence of triterpenes and tannins, while *C.aurantifolin* revealed presence of sterols, flavonoides, tannins, cyanogenic glycosides and coumarins (table 3-2).

(Table 3-1) Yield percentage of the study plants

Study plant	Extract	Initial weight(g)	Final weight(g)	Yield (%)
Cicer arientinum	Aqueous Alcoholic	100 100	0.56 1.44	0.7 1.4
Cinnamomum verum	Aqueous Alcoholic	100 100	1.04 2.55	1.0 2.6
Citrus aurantifolin	Aqueous Alcoholic	50 50	2.65 5.33	5.3 10.7

(Table 3-2) Phytochemical Screening of the Study Plants

Chemical Ingredient	*Cicer arientinum*	*Cinnamomum verum*	*Citrus aurantifolin*
Sterols	+	-	++
Triterpenes	-	+	-
Alkaloids	+	+	+
Flavonoides	-	-	+
Tannins	-	+	+
Anthraquinones	-	-	-
Saponin	++	+	+
Cyanogenic glycosides	++	-	+
Coumarins	++	-	+

3-3. Non Insulin Dependent Diabetes Mellitus (Type II)

3-3-1.*Cicer arientinum*

3-3-1-1. Effects of the aqueous extract of *C.arientinum* on blood glucose , cholesterol and triglycerides of hyperglycaemic rats.

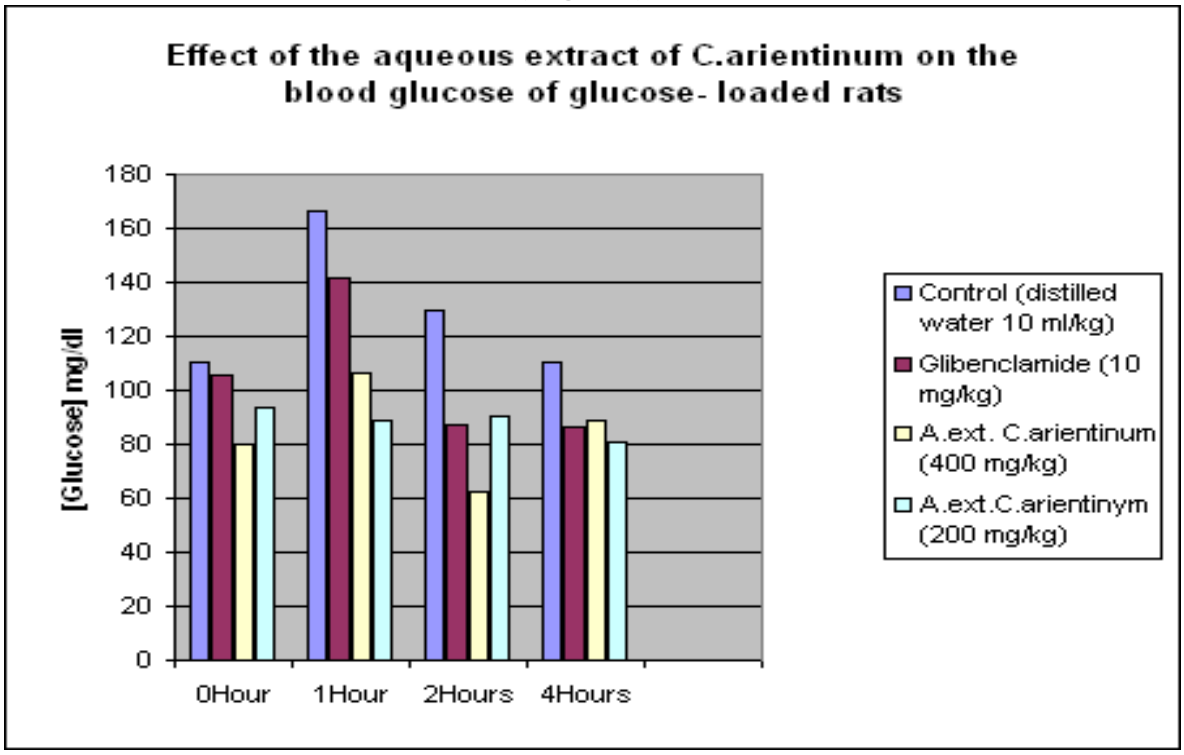
In studying the effect of the aqueous extract of *C.arientinum* on blood glucose level of hyperglycaemic rats, the two doses (400 and 200 mg/kg) showed significant hypoglycaemic effect ($P<0.001$) 1 and 2 hours after extract administration, as compared to the control. The effect continued but with a lesser significance ($P<0.05$).The glucose lowering effect of the reference drug Glibenclamide was not significant, as demonstrated in table (3-3) and figure (3-1). Regarding the extract effect on blood cholesterol, dose 400mg/kg started a highly significant reduction ($P<0.001$) at the first hour post dosing and ($P<0.05$) at the second hour, while dose 200mg/kg started a significant reduction ($P<0.05$) at the first and continued with an increased significant reduction ($P<0.001$), two hours post dosing; (table 3-4). The same effect was revealed on blood triglycerides two hours post dosing as compared to the control, (table 3-5).

3-3-1-2. Effects of the methanolic extract of *C.arientinum* on blood glucose, cholesterol and triglycerides of hyperglycaemic rats.

Compared to the control, dose 400mg/kg expressed a significant reduction ($P<0.05$) on blood glucose level since the 1st hour, the effect continued till the 4th hour. Dose 200mg/kg reduced blood glucose significantly ($P<0.001$), ($P<0.001$) and ($P<0.05$) 1, 2 and 4 hours respectively post dosing. The reference drug, Glibenclamide showed a significant reduction ($P<0.05$), at the 2nd hour, as illustrated in table (3-6). Regarding blood lipids, both doses lowered the level of blood cholesterol

and triglycerides, significantly ($P<0.001$) since the first hour of extract administration, (tables 3-7 and 3-8).

(Fig.3-1)



(Table 3-3) Effect of the aqueous extract of *C.arientinum* on the blood glucose of hyperglycaemic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	110± 8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide (10 mg/kg)	105±5.8	141.7±32.8	87.38±2.89*	86.7±10.45*
C. arientinum (400 mg/kg)	80.98±4.4	106.2±13.6**	62.3±4.27**	88.8±2.45*
C. arientinum (200 mg/kg)	93.7±3.7	88.4±1.25**	90.2±2.3**	80.7±1.25*

(Data are expressed in mean± standard error of mean)

* = ($P<0.05$),

** = ($P<0.001$)

(Table 3-4) Effect of the aqueous extract of *C.arientinum* on the blood cholesterol of hyperglycaemic rats:

Name Of Group	Blood Cholesterol (mg/dl)			
	Time (hours)			
	0	1	2	4
Control (D.W.)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide (10 mg/kg)	88±8.87	87±8.2	83.6±2.8	86.4±21.2
C. arientinum (400 mg/kg)	92±22.5	80.2±3.5**	78.6±5.3*	75.8±11.86
C. arientinum (200 mg/kg)	62.8±6	73±6.2*	72.6±6.4**	79±4.6

(Data are expressed in mean± standard error of mean)

* = (P<0.05).

** = (P<0.001).

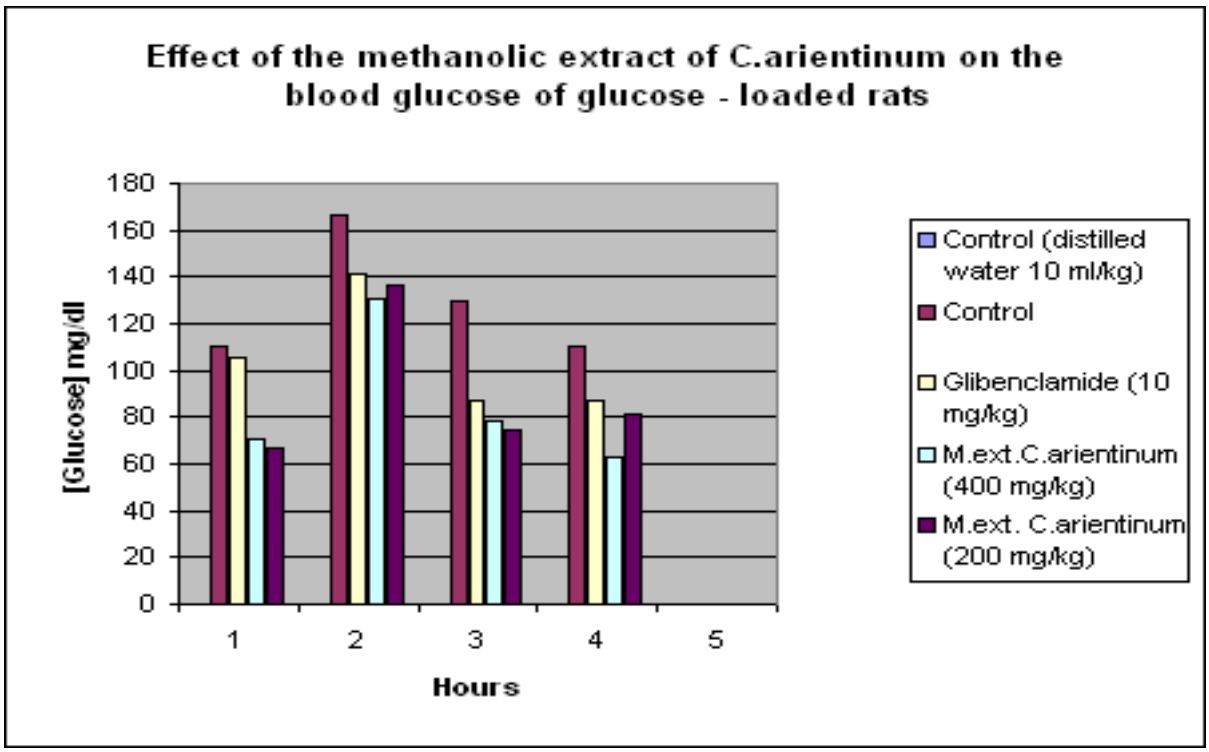
(Table 3-5) Effect of the aqueous extract of *C. arientinum* on the blood triglycerides of hyperglycaemic rats:

Name Of Group	Blood Triglycerides (mg/dl)			
	Time (hours)			
	0	1	2	4
Control (distilled water 10 ml/kg)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide (10 mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C. arientinum (400 mg/kg)	146.8±12.4	153.6±7.5	129.6±5.9*	140.6±11.8
C. arientinum (200 mg/kg)	145.2±13.9	121.6±12.4	117.2±6.5*	150.2±10.6

(Data are expressed in mean ± standard error of mean)

* = (P<0.05).

(Fig. 3-2)



(Table 3-6) Effect of the methanolic extract of *C. arientinum* on the blood glucose of hyperglycaemic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	110±8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide (10 mg/kg)	105±5.8	141.7±32.8	87.38±2.89*	86.7±10.45
C. arientinum (400 mg/kg)	71±1.84	131±5*	87.5±1.74*	72.8±1.65*
C. arientinum (200 mg/kg)	66.6±2.37	136±7.2**	74.8±1.46**	81±17.2*

(Data are expressed in mean± standard error of mean)

* = ($P<0.05$),

* * = ($P<0.001$),

(Table 3-7) Effect of the methanolic extract of *C. arientinum* on the blood cholesterol of hyperglycaemic rats:

Name Of Group	Blood Cholesterol (mg/dl)			
	Time (hours)			
	0	1	2	4
Control (D.W.)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide (10 mg/kg)	88±8.87	87±8.2	83.6±2.8	86.4±21.2
C. arientinum (400 mg/kg)	55.2±1.6*	66±4.7**	56.8±1.3**	51.2±2.7**
C. arientinum (200 mg/kg)	71.4±3.1	74.6±8.3**	65.4±1.9**	55.6±6.1**

(Data are expressed in mean± standard error of mean)

** = (P<0. 001).

(Table 3-8) Effect of the methanolic extract of *C. arientinum* on the blood triglycerides of hyperglycaemic rats:

Name Of Group	Blood Triglycerides (mg/dl)			
	Time (hours)			
	0	1	2	4
Control (D.W.)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide (10 mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C. arientinum (400 mg/kg)	125±8.6	72±4.1**	54.2±1.7**	65.8±1.5**
C. arientinum (200 mg/kg)	122.4±3.2	60±1.8**	56.6±4.2**	66.2±10.5**

(Data are expressed in mean± standard error of mean)

** = (P<0. 001).

3-3-2 *Cinnamomum verum*

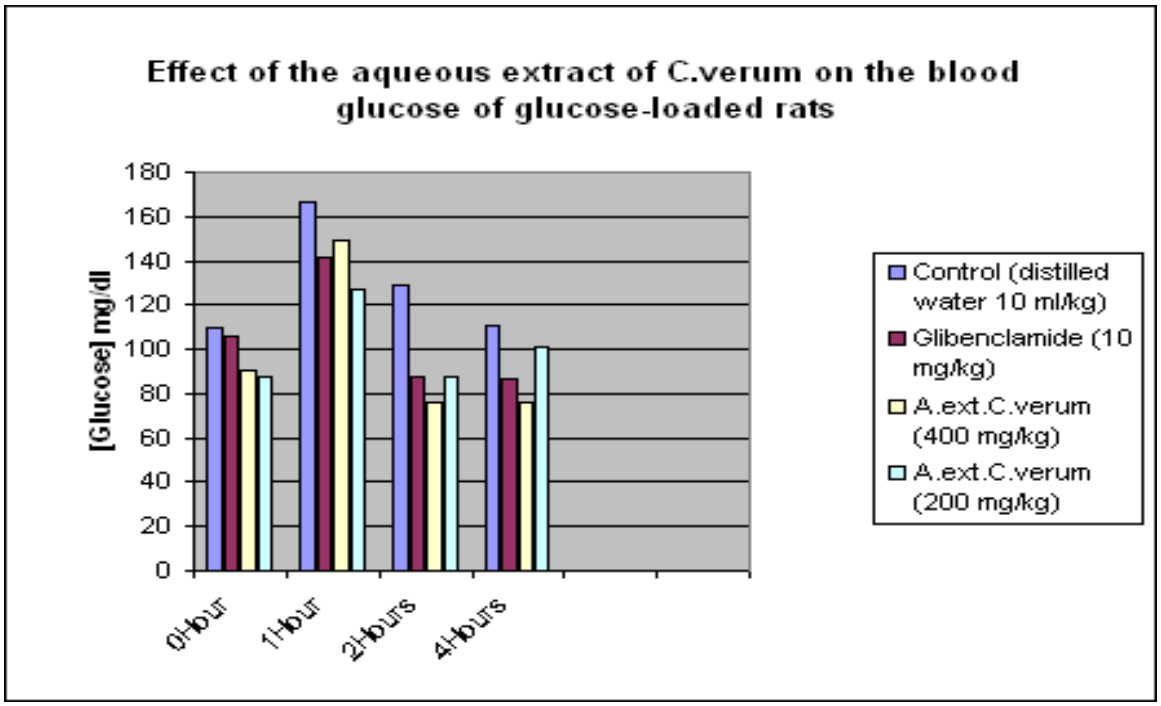
3-3-2-1. Effects of the aqueous extract of *C.verum* on blood glucose cholesterol and triglycerides of hyperglycaemic rats.

In studying the effect of the aqueous extract of *C.verum* on blood glucose level of hyperglycaemic rat**s,** the two doses (400 and 200 mg/kg) showed a significant hypoglycaemic effect (P<0. 001) since the first hour of dose administration as shown in table (3-9). The hypoglycaemic effect of the reference drug Glibenclamide was not significant as compared to the control. Regarding its effect on blood lipids, neither blood cholesterol nor blood triglycerides were significantly reduced by the extract, (tables 3-10 & (3-11).

3-3-2-2. Effects of the methanolic extract of *C.verum* on blood glucose cholesterol and triglycerides of hyperglycaemic rats.

Compared to the control, dose (400 mg/kg) showed a significant reduction (P<0. 001) in the level of blood sugar, one hour after administration of the extract, this effect continued but with a lesser significance (P<0.05). Table (3-12) and Fig (3-4). illustrate the hypoglycaemic effect of dose (200 mg/kg) which was highly significant (P<0. 001). Regarding the effect on blood cholesterol, there was no significant reduction, as compared to the control (table 3-13). With respect to blood triglycerides, the two doses of the methanolic extract of *C.verum* produced a significant reduction (P<0.05) 2 and 4 hours post dosing, (table 3-14).

(Fig. 3-3)



(Table 3-9) Effect of the aqueous extract of *C.verum* on the blood glucose of hyperglycaemic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	110±8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide (10 mg/kg)	105±5.8	141.7±32.8	87.38±2.89*	86.7±10.45
C. verum (400 mg/kg)	73.9±9.9	93.5±1.9**	92.7±2**	87.5±9.7**
C. verum (200 mg/kg)	93.6±7.5	88.8±7.9**	85±2.3**	72.5±4.1**

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001)

(Table 3-10) Effect of the aqueous extract of *C. verum* on the blood cholesterol of hyperglycaemic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide (10 mg/kg)	88±8.87	87±8.2	83.6±2.8	86.4±21.2
Cverum (400 mg/kg)	69.4±5.1	111.8±20.5	92.6±17.9	86.7±1.4
Cverum (200 mg/kg)	70.2±3.7	72.8±4.6	90.4±1.8	80.6±6.9

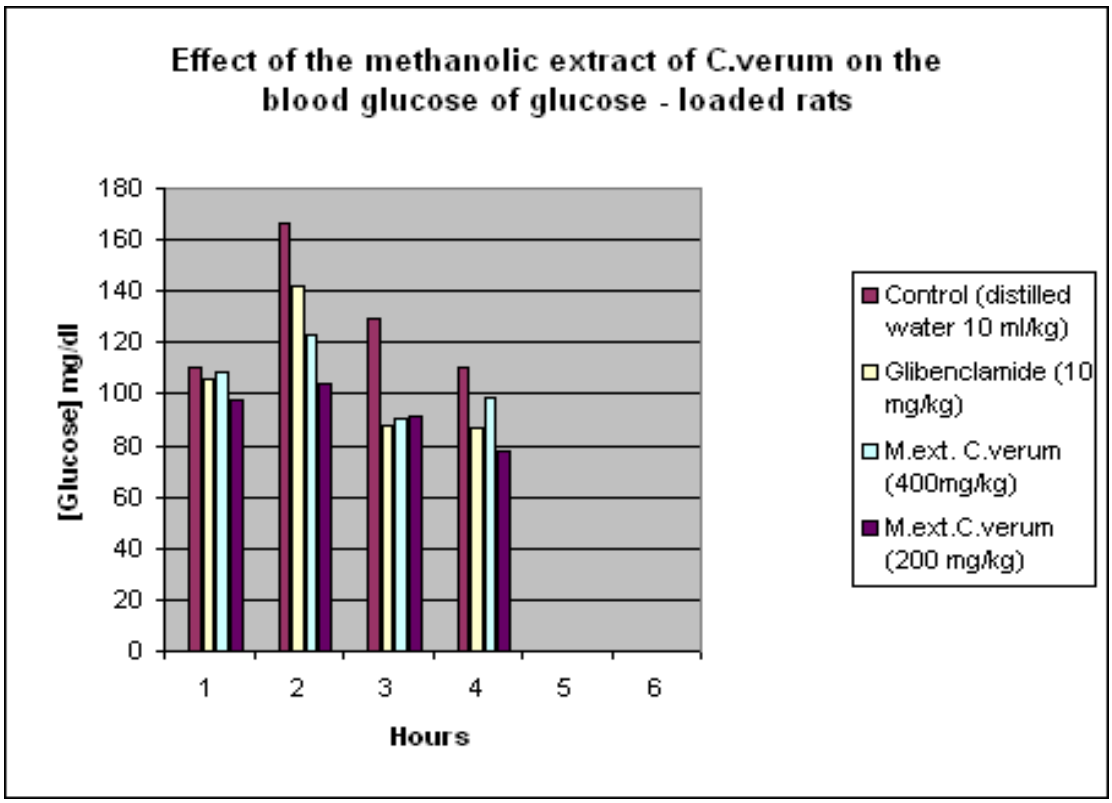
(Data are expressed in mean± standard error of mean)

(Table 3-11) Effect of the aqueous extract of *C. verum* on the blood triglycerides of hyperglycaemic rats:

Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide (10 mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C. arientinum (400 mg/kg)	138.4±11	144±4.5	121±14.2	115±10.
C. arientinum (200 mg/kg)	131.2±10.9	129.2±6	136.8±8.5	120.2±7.5

(Data are expressed in mean± standard error of mean)

(Fig. 3-4)



(Table 3-12) Effect of the methanolic extract of *C. verum* on the blood glucose of hyperglycaemic rats

Name Of Group	Blood Glucose (mg/dl)			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	110±8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide (10 mg/kg)	105±5.8	141.7±32.8	87.38±2.89	86.7±10.45
C. verum (400 mg/kg)	108.6±8.3	122.5±1.2**	90.6±16.2*	90.7±11.2*
C. verum (200 mg/kg)	97.6±7.6	104.2±15**	91.2±7.4**	78±5.5**

(Data are expressed in mean± standard error of mean)

* = ($P<0.05$),

** = ($P<0.001$)

(Table 3-13) Effect of the methanolic extract of *C. verum* on the blood cholesterol of hyperglycaemic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide (10 mg/kg)	88±8.87	87±8.2	83.6±2.8	86.4±21.2
C. arientinum (400 mg/kg)	69.4±5.1	92.6±17.9	101.8±20.5	86.7±1.4
C. arientinum (200 mg/kg)	70.2±3.7	72.8±4.6	90.4±1.8	80.6±6.9

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001)

(Table 3-14) Effect of the methanolic extract of *C. verum* on the blood triglycerides of hyperglycaemic rats:

Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide (10 mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C. arientinum (400 mg/kg)	138.4±11	124±4.5	111±14.2*	105±10.*
C. arientinum (200 mg/kg)	131.2±10.9	129.2±6	116.8±8.5*	108.2±7.5*

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001)

3-3-3. *Citrus aurantifolin*

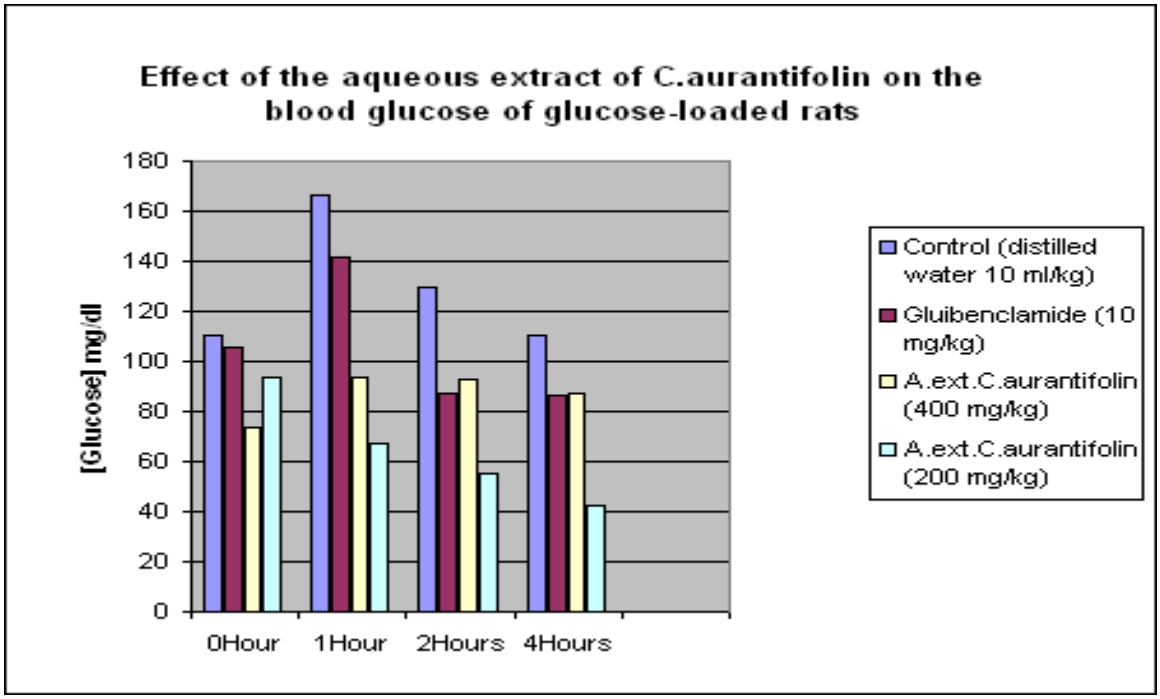
3-3-3-1. Effects of the aqueous extract of *C.aurantifolin*on blood glucose cholesterol and triglycerides of hyperglycaemic rats.

In studying the effect of the aqueous extract of *C.aurantifolin,* on blood glucose level of hyperglycaemic rat**s,** both doses, reduced blood glucose significantly (P<0.05), (P<0. 001) and (P<0. 001) 1, 2 and 4 hours post dosing respectively. Table (3-15), illustrates that the reference drug Glibenclamide showed no significant reduction as compared to the control. Table (3-16), reveals that, both doses of the aqueous extract of *C. aurantifolin*, reduced blood cholesterol significantly (P<0.001) 1 hour post dosing. The effect of dose (200 mg/kg) was more persistent. Regarding blood triglycerides, dose (400 mg/kg) revealed a significant reduction (P<0. 001) 2 and 4 hours post dose administration, as demonstrated in table (3-17).

3-3-3-2. Effects of the methanolic extract of *C.aurantifolin*on blood glucose cholesterol and triglycerides of hyperglycaemic rats.

Dose (400 mg/kg) revealed a significant reduction in blood glucose (P<0. 001) and (P<0.05), one and two hours post dosing, respectively. Dose (200 mg/kg) showed a significant reduction (P<0.05), one hour after administration of extract. The reference drug Glibenclamide revealed a significant reduction (P<0.05).two hours post dosing, as illustrated in table (3-18). Regarding the effect on blood lipids**,** the two doses showed a reduction on the level of blood cholesterol. The reference drug Glibenclamide revealed a significant reduction (P<0.05), two hours post dosing as illustrated in table (3-19). The two doses of the methanolic extract of *C.aurantifolin*, as demonstrated in table (3-20), showed a non significant reduction on the level of blood triglycerides of hyperglycaemic rats.

(Fig.3-5)



(Table 3-15) Effect of the aqueous extract of *C.aurantifolin* on the blood glucose of hyperglycaemic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	110±8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide (10 mg/kg)	105±5.8	141.7±32.8	87.38±2.89*	86.7±10.45
C. aurantifolin (400 mg/kg)	90±10.6	149.5±9.1*	76±10.5**	75.9±6.9**
C. aurantifolin (200 mg/kg)	87.8±2.7	127±11.3*.	87.2±4.8**	100.9±0.73*

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001)

(Table 3-16) Effect of the aqueous extract of *C.aurantifolin* on the blood cholesterol of hyperglycaemic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide (10 mg/kg)	88±8.87	87±8.2	83.6±2.8*	86.4±21.2
C. arientinum (400 mg/kg)	66.6±4.8	67.8±9.6**	107.2±13.2	83.5±6.4
C. arientinum (200 mg/kg)	57.4±4.5	71.8±5.6**	68.6±4.4**	67.7±6.8**

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001)

(Table 3-17) Effect of the aqueous extract of *C.aurantifolin* on the blood triglycerides of hyperglycaemic rats:

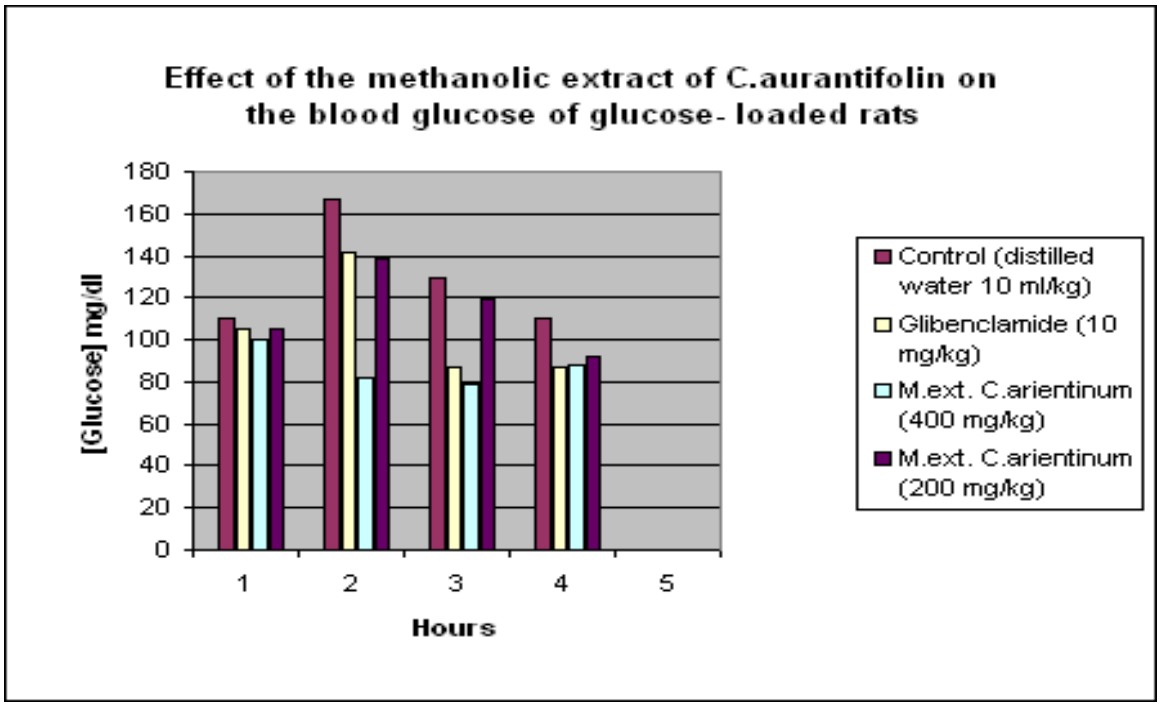
Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (water 10 ml/kg)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide (10 mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C. arientinum (400 mg/kg)	115.4±16.7	135.2±19.4	87±22.4**	74±20**
C. arientinum (200 mg/kg)	131±10.5	115.2±9.7	118.4±5.3*	108.5±14.9

(Data are expressed in mean± standard error of mean)

* = (P<0.05)

** = (P<0. 001).

(Fig.3-6)



(Table 3-18) Effect of the methanolic extract of *C. aurantifolin* on the blood glucose of hyperglycaemic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	110± 8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide (10 mg/kg)	105±5.8	141.7±32.8	87.38±2.89	86.7±10.45
C. aurantifolin (400 mg/kg)	100.2±2.4	82.2**±15	76.4±3.6*	88.5±8.8
C. aurantifolin (200 mg/kg)	105±8.7	138.2±2.1*	119.1±6.7	91.9±1.6

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001).

(Table 3-19) Effect of the methanolic extract of *C. aurantifolin* on the blood cholesterol of hyperglycaemic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide (10 mg/kg)	88±8.87	87±8.2	83.6±2.8	86.4±21.2
C. arientinum (400 mg/kg)	72.6±5.1	104.2±16.8	93.2±21.6	75.8±4.6
C. arientinum (200 mg/kg)	65±3.7	79.8±21.6*	88.2±6.4*	80±6

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001).

(Table 3-20) Effect of the methanolic extract of *C. aurantifolin* on the blood triglycerides of hyperglycaemic rats:

Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**1**	**2**	**4**
Control (D.W.)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide (10 mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C. arientinum (400 mg/kg)	123.8±9.7	131.2±5.6	120.8±9.5	114.4±7.5
C. arientinum (200 mg/kg)	125.4±19.2	114±22.4	101.4±11.8	99.4±16.3

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001).

3-4. Insulin-Dependent Diabetes Mellitus

(Type I)

3-4-1. *Cicer arientinum*

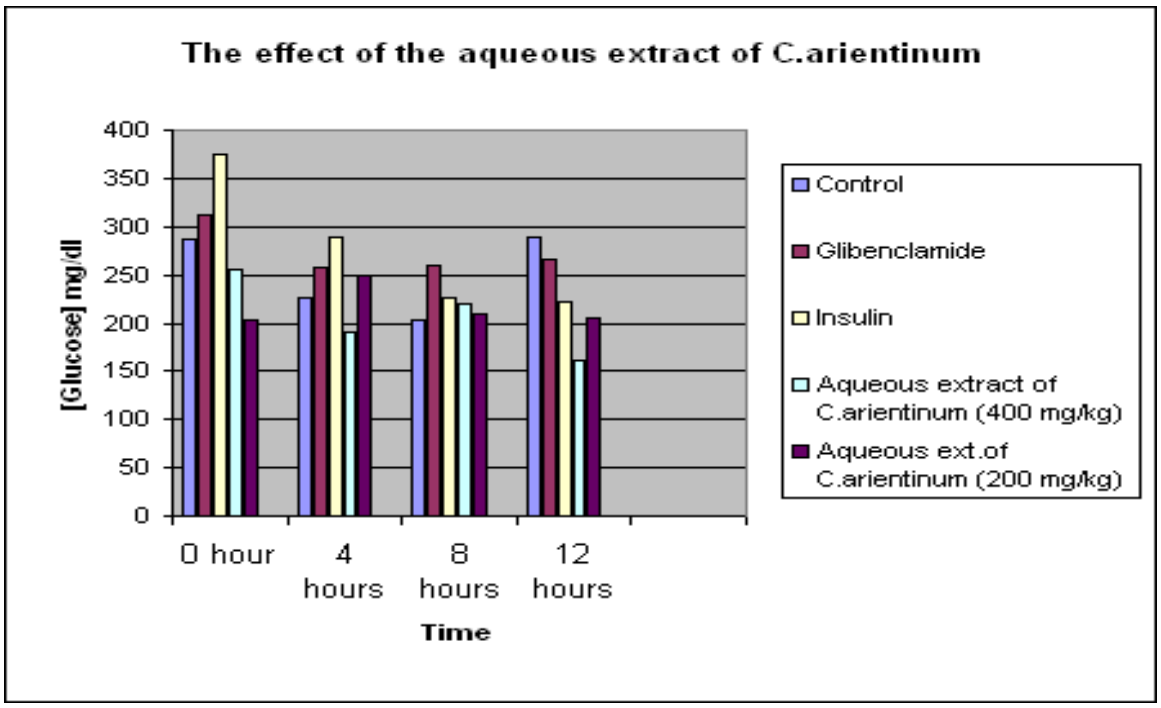
3-4-1-1. Effects of the aqueous extract of *C.arientinum*on blood glucose cholesterol and triglycerides of diabetic ats.

Compared to the control dose 400 mg/kg of the aqueous extract of *C.arientinum*, revealed a significant hypoglycaemic effect ($P<0.05$) throughout the experiment. The effect was more significant ($P<0.001$) at the 12th hour post extract administration. The smaller dose (200 mg/kg) revealed a slower but highly significant effect ($P<0.001$).The reference drugs Glibenclamide and Insulin, revealed no significant hypoglycaemic effects as shown in table (3-21). Regarding blood cholesterol, the two doses (400 and 200 mg/kg) of the aqueous extract of *C.arientinum* exerted no significant effect. Table (3-22), demonstrates that, Insulin exerted a significant reduction ($P<0.05$), four and eight hours respectively, after dosing. Regarding blood triglycerides, dose (400 mg/kg) showed a significantl reduction ($P<0.001$), twelve hours after administration of the extract. Dose 200mg/kg showed a more persistent and significant lowering effect ($P<0.05$), ($P<0.05$) and ($P<0.001$) respectively 4, 8 and 12 hours after extract administration. Insulin produced a significant reduction ($P<0.001$), four and eight hours respectively, after dosing, as demonstrated in table (3-23).

3-4-1-2. Effects of the methanolic extract of *C.arientinum*on blood glucose cholesterol and triglycerides of diabetic ats.

As illustrated in table (3-24), both doses of the methanolic extract of *C.arientinum*, produced a significant hypoglycaemic activity ($P<0.001$), twelve hours after administration of the extract, as compared to the contro. The reference Glibenclamide revealed no significant effect, while Insulin exerted a significant reduction ($P<0.05$), at the 12^{th} hour, post dosing. Regarding its effect on blood lipids, none of the doses reduced blood cholesterol significantly table (3-25). Table (3-26), reveals that, dose (400 mg/kg) exerted a significant lowering effect ($P<0.001$) on the level of blood triglycerides of diabetic rats after twelve hours from extract administration. Similar to Glibenclamide, dose 200 mg/kg showed no lowering effect on blood triglycerides, while Insulin reduced blood triglycerides significantly ($P<0.001$) 4, 8, and 12 hours post administration.

(Fig.3-7)



(Table 3-21) Effect of the aqueous extract of *C.arientinum* on the blood glucose of diabetic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide (10 mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble Insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2.8	222±6.6
C. *arientinum* (400 mg/kg)	255.6±14.8	199.2±16.4*	190.6±26.5*	162±27.6**
C. *arientinum(*200 mg/kg)	211±16.6	249.2±23.8	208.8±25.3	205.4±15.1**

(Data are expressed in mean± standard error of mean)

* = (P<0. 05)

** = (P<0. 001)

(Table 3-22) Effect of the aqueous extract of *C.arientinum* on the blood cholesterol of diabetic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide (10 mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble Insulin (3U/kg)	40.4±2.5	31.4±3*	34.2±.8*	42±2.1
C. *arientinum* (400 mg/kg)	75.6±12.3	71±14.5	70±15.5	54.8±5.2
C. *arientinum* (200 mg/kg)	77.4±5.1	65±17.7	94.4±20.8	57.8±11.2

(Data are expressed in mean± standard error of mean)

* = (P<0.05)

(Table 3-23) Effect of the aqueous extract of *C.arientinum* on the plasma triglycerides level of diabetic rats:

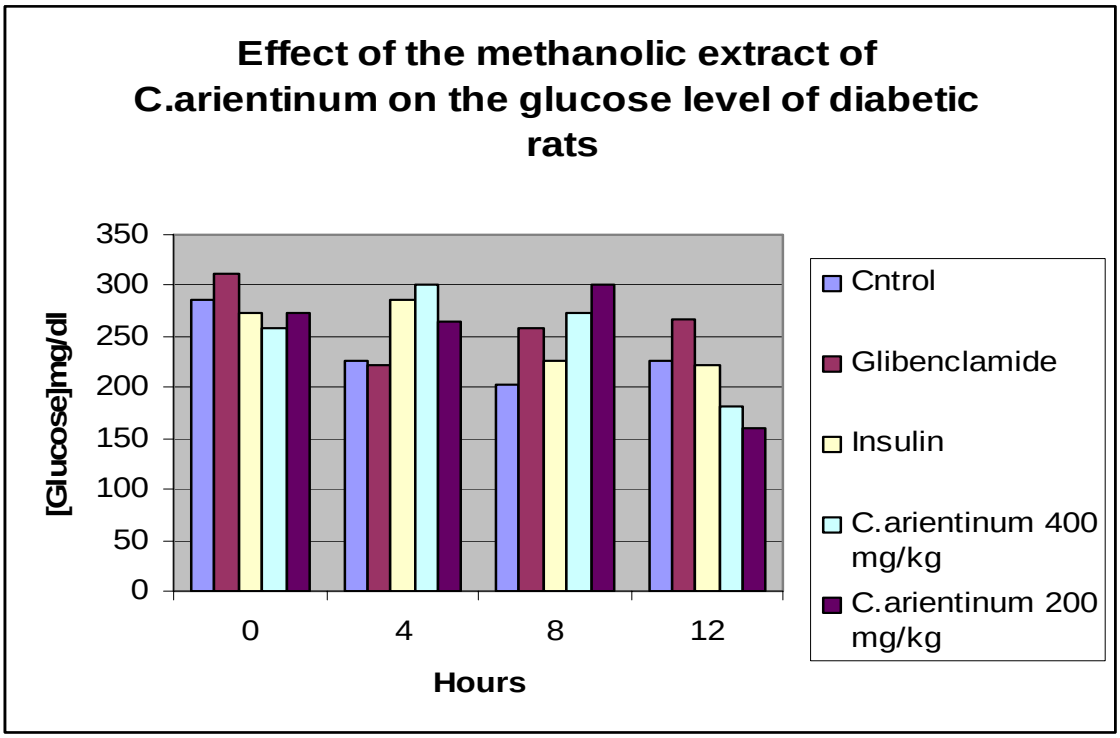
Name Of Group	**Blood Triglycerides (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	247.2±35.7	227.8±20.3	187±15.3	284±54.3
Glibenclamide (10 mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble Insulin (3 U/kg)	57.8±12.8	33.2±4.8**	33.6±2.5**	242±6.5
C. arientinum (400 mg/kg)	225.6±18.7	239.8±125.3	206.2±104.7	106±58.5**
C. arientinum (200 mg/kg)	190.4±78.8	176.8±40.3*	168.4±107*	75±12.6**

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001)

(Fig.3-8)



(Table 3-24) Effect of the methanolic extract of *C.arientinum* on the blood glucose of diabetic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide (10 mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble Insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6
C. aurantifolin (400 mg/kg)	258±12.9	300.4±10.7	274.2±30.9	181±3.7**
C. aurantifolin (200 mg/kg)	274.8±13.4	264.8±38.6	300.4±16.3	161.6±32.4**

(Data are expressed in mean± standard error of mean)

** = (P<0. 001)

(Table 3-25) Effect of the methanolic extract of *C.arientinum* on the blood cholesterol of diabetic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide (10 mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble Insulin (3U/kg)	40.4±2.5	31.4±3	34.2±.8	42±2.1
C. arientinum (400 mg/kg)	67±8.8	71.8±21	63.4±16	40.8±4.4
C. arientinum (200 mg/kg)	78.6±15.6	57±12	76.4±15.2	66.3±10.2

(Data are expressed in mean± standard error of mean)

(Table 3-26) Effect of the methanolic extract of *C.arientinum* on the blood triglycerides of diabetic rats:

Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	247.2±35.7	227.8±20.3	187±15.3	284±54.3
Glibenclamide (10 mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble Insulin (3U/kg)	57.8±12.8	33.2±4.8**	33.6±2.5**	42±6.5**
C. arientinum (400 mg/kg)	213.6±20.9	280±102.6	276.6±89	159.8±51.1**
C. arientinum (200 mg/kg)	267.8±36.7	297.4±29.2	285.4±36	238.8±24.9

(Data are expressed in mean± standard error of mean)

* = ($P<0.05$),

** = ($P<0.001$)

3-4-2 Cinnanomum verum

3-4-1-1. Effects of the aqueous extract of *C.verum* on blood glucose cholesterol and triglycerides of diabetic ats.

Table (3-27), reveals that, dose (400 mg/kg) reduced blood glucose level of diabetic rats significantly (P<0.05), (P<0.001), 8 and 12 hours post dosing respectively, as compared to the control.. Dose (200 mg/kg) exerted a significant hypoglycaemic effect (P<0. 001) after 12 hours. Neither Glibenclamide nor insulin reduced blood glucose level of diabetic rats. The results are illustrated on (table 3-28), show that, neither *C. verum*, nor Insulin, produced a significant reduction on the blood level of diabetic rats diabetic rats. Regarding blood triglycerides, both doses of the aqueous extract of *C.verum* produced a significant lowering effect (P<0. 001) 8 and 12 hours post dosing. Table (3-29), shows that, the lowering effect exerted by insulin has an early onset as it started since the 4 th hour post dosing.

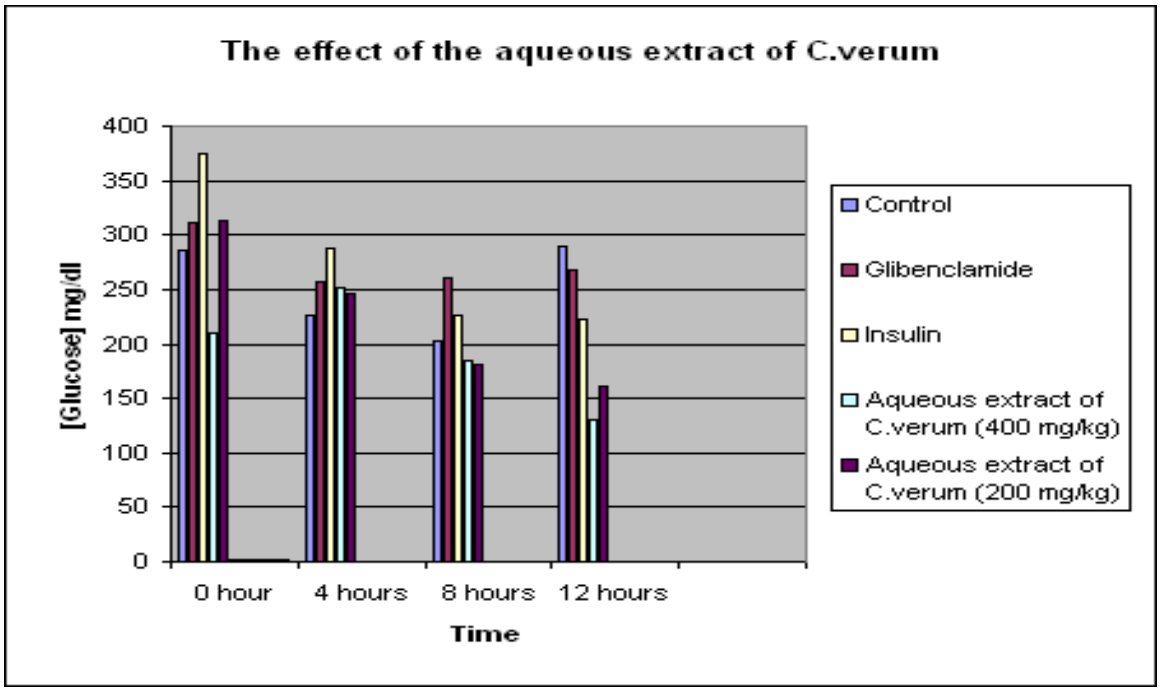
3-4-1-2. Effects of the methanolic extract of *C.verum* on blood glucose cholesterol and triglycerides of diabetic ats.

Both doses of the methanolic extract of *C.verum* produced a slow but highly significant reduction on the glucose blood level of diabetic rats at the 12 th hour post extract administration. Neither Glibenclamide nor insulin revealed a significant hypoglycaemic effect on diabetic rats as shown on (table 3-30).

Regarding blood cholesterol, *C.verum*, showed no significant reduction on the blood cholesterol level of diabetic rats, while Insulin produced a significant reduction (P<0. 05), four hours after dosing, as demonstrated in (table 3-31). Both doses of the methanolic extract of *C.verum* as well as the reference drug (insulin) showed a significant lowering effect (P<0. 001) on the level of blood triglycerides. The onset of the effect of insulin

was earlier as it started since the 4th hour post dosing while the effect of the extract appeared at the 12th hour, table (3-32).

(Fig 3-9)



(Table 3-27) Effect of the aqueous extract of C. verum on the blood glucose of diabetic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide (10 mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble Insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6
C.verum (400 mg/kg)	303±7.2	254.4±23.4	182.4±62.8*	156.2±42.2**
C.verum (200 mg/kg)	293.8±7.7	269.2±45.1	202.8±31.5	186±18.7**

Data are expressed in mean ± standard error of mean

* = ($P<0.05$)

** = ($P<0.001$)

(Table 3-28) Effect of the aqueous extract of *C. verum* on the blood cholesterol of diabetic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide (10 mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble Insulin (3U/kg)	40.4±2.5	31.4±3	34.2±.8	42 ±2.1
C.verum (400 mg/kg)	95.8±6.5	79±20.6	50.6±6.7	50±1.6
C.verum (200 mg/kg)	100.4±53	73.2±13.8	58.6±19.3	54.4±19.2

(Data are expressed in mean ± standard error of mean)

* = (P<0.05).

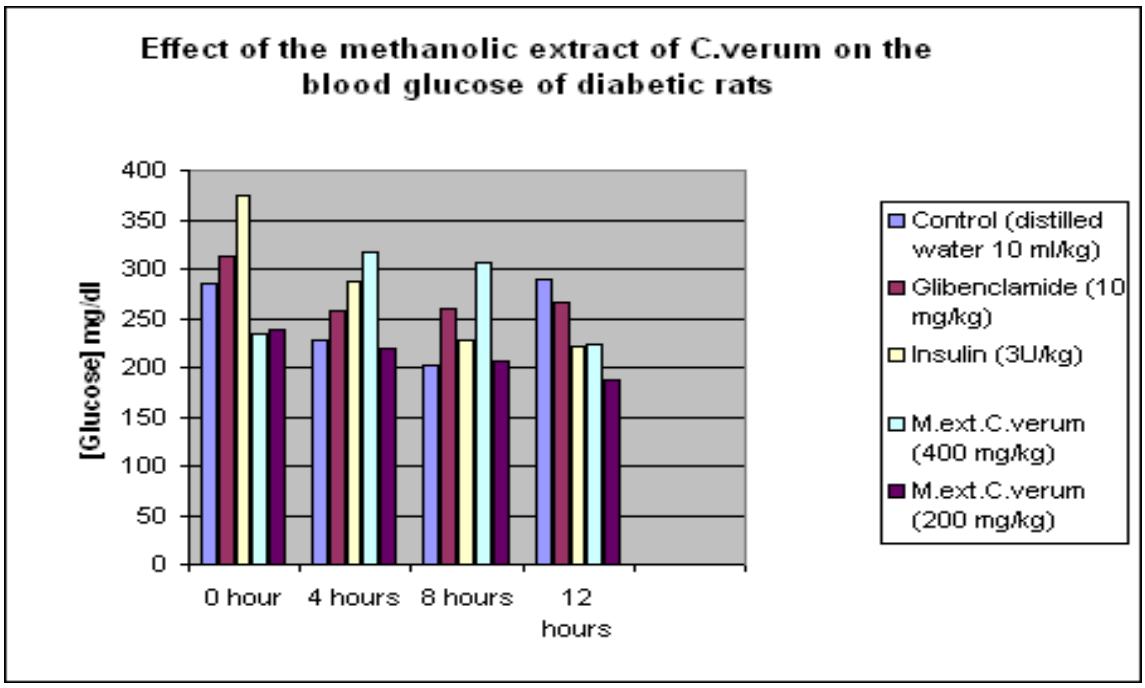
(Table 3-29) Effect of the aqueous extract of *C. verum* on the blood triglycerides of diabetic rats:

Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	247.2±35.7	227.8±20.3	187±15.3	284±54.3
Glibenclamide (10 mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble Insulin (3U/kg)	57.8±12.8	33.2±4.8**	33.6±2.5**	42±6.5**
C.verum (400 mg/kg)	285.8±34.8	305±47.1	88.6±19.2**	165±64.7**
C.verum (200 mg/kg)	186.8±65.7	227.8±20.3	88.2±29.5**	88.6±28**

(Data are expressed in mean± standard error of mean)

** = (P<0. 001).

(Fig. 3-10)



(Table 3-30) Effect of the methanolic extract of *C. verum* on the blood glucose of diabetic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide (10 mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble Insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6
C.verum (400 mg/kg)	235.4±12.4	315.6±31.9	206.2±54.1	203.6±57.1**
C.verum (200 mg/kg)	239.4±15.4	220.4±25.9	205.6±32.3	187.4±41.7**

(Data are expressed in mean± standard error of mean)

* = ($P<0.05$),

** = ($P<0.001$).

(Table 3-31) Effect of the methanolic extract of *C. verum* on the blood cholesterol of diabetic rats**:**

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide (10 mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble Insulin (3U/kg)	40.4±2.5	31.4±3	34.2±.8	42±2.1
C.verum (400 mg/kg)	58.4±8.2	51.8±7.1	45.2±5.9	42.6±1.7
C.verum (200 mg/kg)	79±3.4	48.6±5.1	52.8±2.5	47.8±2.9

(Data are expressed in mean± standard error of mean)

(Table 3-32) Effect of the methanolic extract of *C. verum* on the blood triglycerides of diabetic rats:

Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	247.2±35.7	227.8±20.3	187±15.3	284±54.3
Glibenclamide (10 mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble Insulin (3U/kg)	57.8±12.8	33.2±4.8**	33.6±2.5**	42±6.5**
C.verum (400 mg/kg)	210.8±51.2	283.4±16.2	165.2±22.6	165.8±43.7**
C.verum (200 mg/kg)	179.8±65	195±57.9	223.2±61.4	165.2±43.1**

(Data are expressed in mean± standard error of mean)

** = ($P<0.01$)

3-4-3- *Citrus aurantifolin*

3-4-3-1- Effects of the aqueous extract of *C.aurantifolin* on blood glucose cholesterol and triglycerides of diabetic ats.

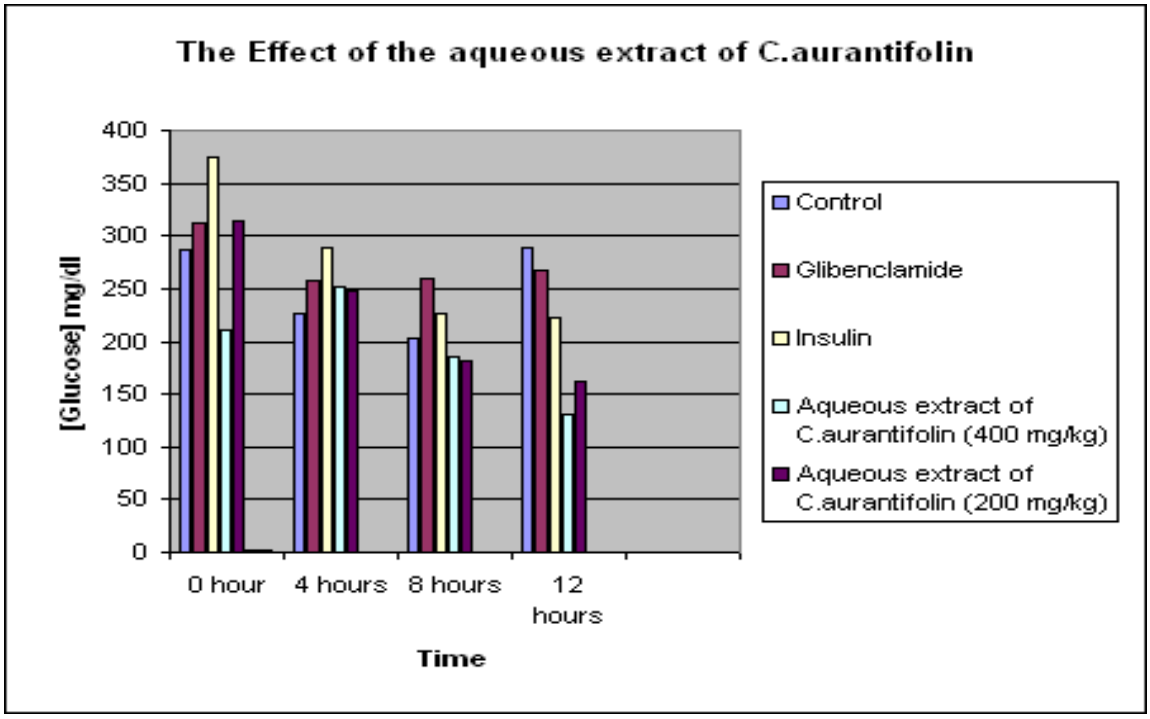
Compared to the control, both doses (400 and 200 mg/kg) of the aqueous extract of *C.aurantifolin* reduced the level of blood glucose of diabetic rats significantly (P<0.05), (P<0.001), 4 and 8 hours after extract administration respectively. The hypoglycaemic effect exerted by the extract was greater than that produced by the reference drugs Glibenclamide and Insulin, as illustrated in (table 3-33). Regarding blood cholesterol**,** table (3-34). Illustrates that, both doses of the aqueous extract of *C.aurantifolin*, showed a non significant reduction in diabetic rats, while Insulin exerted a significant reduction (P<0. 05) at the 4^{th} hour post dosing. Regarding blood triglycerides, table (3-35), shows that, the two doses, similar to Insulin, reduced the level of blood triglycerides significantly (P<0. 001) throughout the 12 hours of the experiment, while Glibenclamide exerted a slight and slow significant reduction (P<0. 05) at the 12^{th} hour post dosing.

3-4-3-2- Effects of the methanolic extract of *C.aurantifolin* on blood glucose cholesterol and triglycerides of diabetic ats.

Table (3-36), demonstrates that, both doses exerted a significant hypoglycaemic effect (P<0.001), in diabetic rats 12 hours after extract administration. Neither Glibenclamide nor insulin reduced blood glucose. Regarding blood cholesterol, *C.aurantifolin*, showed no significant reduction diabetic rats, while Insulin revealed a significant reduction (P<0. 05) at the 4^{th} hour post dosing, as shown in table (3-37). Regarding blood triglycerides, both doses, reduced the level of blood triglycerides of diabetic rats significantly (P<0.05), (P<0. 001) 8 and 12 hours post extract administration. The reference drug insulin produced a highly

significant reduction (P<0. 001), since the 4th hour and the effect persisted till the 12th hour. Glibenclamide showed no effect, (table 3-38).

(Fig.3-11)



(Table 3-33) Effect of the aqueous extract of *C. aurantifolin* on the blood glucose of diabetic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide (10 mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble Insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6
C. aurantifolin (400 mg/kg)	210.4±19.5**	249.6±20.2	184.8±2.2*	130.8±2.2**
C. aurantifolin (200 mg/kg)	314±44	247.2±52.3	180.6±14.4*	160.6±5**

Data are expressed in mean± standard error of mean

* = ($P<0.05$),

** = ($P<0.001$).

(Table 3-34) Effect of the aqueous extract of *C. aurantifolin* on the cholesterol of diabetic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide (10 mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble Insulin (3U/kg)	40.4±2.5	31.4±3*	34.2±.8	42±2.1
C.aurantifolin (400 mg/kg)	75.8±12.7	51±4.7	39.6±3.3	42.8±1.1
C.aurantifolin (200 mg/kg)	65.6±13.7	87.4±13	48.8±.8	47.2±.7

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

(Table 3-35) Effect of the aqueous extract of *C. aurantifolin* on the blood triglycerides of induced- diabetic rats:

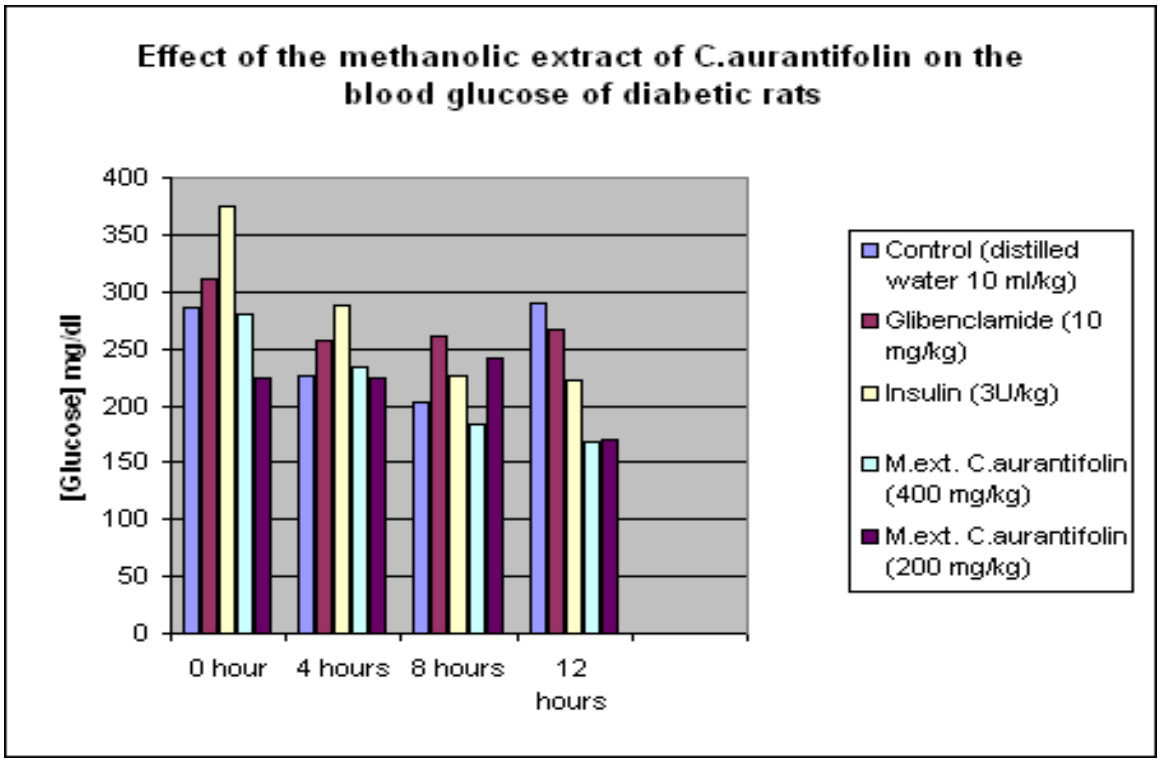
Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	247.2±35.7	227.8±20.3	187±15.3	284±54.3
Glibenclamide (10 mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5*
Soluble Insulin (U/kg)	57.8±12.8	33.2±4.8**	33.6±2.5**	42±6.5**
C.aurantifolin (400 mg/kg)	122.2±14.3	55.8±12.8**	43.4±8**	27.4±0.8**
C.aurantifolin (200 mg/kg)	143.2±36.6	243±37**	32.4±6.6**	44.4±5.3**

(Data are expressed in mean± standard error of mean)

* = (P<0.05),

** = (P<0. 001).

(Fig. 3-12)



(Table 3-36) Effect of the methanolic extract of *C. aurantifolin* on the blood glucose of induced- diabetic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide (10 mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble Insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6
C. aurantifolin (400 mg/kg)	277.8±22.7	233.2±14.7	179.8±16.6	168.4±8.6**
C. aurantifolin (200 mg/kg)	224.8±10	223.6±6.8	201±41.6	171.4±29.8**

(Data are expressed in mean± standard error of mean)

* = ($P<0.05$),

** = ($P<0.001$).

(Table 3-37) Effect of the methanolic extract of *C. aurantifolin* on the blood Cholesterol of induced- diabetic rats:

Name Of Group	**Blood Cholesterol (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide (10 mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble Insulin (3U/kg)	40.4±2.5	31.4±3*	34.2±.8	42±2.1
C.aurantifolin (400 mg/kg)	58.8±5.9	33±2.7	49.2±5.9	41±5.1
C.aurantifolin (200 mg/kg)	63.4±11	48±5.4	69±5.4	51.2±5

(Data are expressed in mean± standard error of mean)

** = (P<0. 05).

(Table3-38) Effect of the methanolic extract of *C. aurantifolin* on the blood triglycerides of induced- diabetic rats

Name Of Group	**Blood triglycerides (mg/dl)**			
	Time (hours)			
	0	**4**	**8**	**12**
Control (D.W.)	247.2±35.7	227.8±20.3	187±15.3	284±54.3
Glibenclamide (10 mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble Insulin (U/kg)	57.8±12.8	33.2±4.8**	33.6±2.5**	42±6.5**
C.aurantifolin (400 mg/kg)	210.2±51.2	283.4±16.2	165.2±43.7*	156.8±43.7**
C.aurantifolin (200 mg/kg)	179.8±65	195±57.9	165.2±61.4*	163.2±43.1**

(Data are expressed in mean± standard error of mean)

** = (P<0. 05).

* = (P<0. 001).

3-5 Pharmacology

The three plant extracts showed significant relaxant activity when tested on isolated rabbit jejunum in different doses (0.04 µg/ml, 0.08 µg/ml and 0.16 µg /ml). This relaxant activity in the plant extracts of *C.arientinum* and *C.verum* were blocked by different doses of Phentolamine. On the other hand the relaxant activity of the plant extract of *C.aurantifolin* was not blocked by any of blockers as demonstrated on table (3-39).

(Table 3-39): Pharmacological screening of the study plant extracts:

Plant name	Concentration used (mg/ml)	Sub maximum dose (ml)	Response	Blocker
C.arientinum	1	0.8	Relaxation	Phentolamine
C.verum	20	0.8	Relaxation	Phentolamine
C.aurantifn	1	0.8	Relaxation	-

3-6. Toxicology

3-6-1. Sub-chronic toxicity:

The effect of methanolic extracts of *C.arientinum, C.verum* and *C. aurantifolin* on adult healthy rats receiving these plants for 21 days, revealed no significant alterations in blood haemogram, liver functions and kidney functions. The results of the liver functions represented by GOT, GPT and ALP showed no significant alteration as demonstrated in table (3-40). Kidney function tests represented by creatinine, urea, Na and K showed no significant changes, compared to the control as demonstrated in table (3-41). Similarly, the effect of these plants on blood haemogram represented by TWBCs, TRBCs and Hb are shown in table (3-42).

Furthermore, compared to the control, the histopathological investigation of these plants revealed normal hepatocytes as demonstrated in table (3-43).

(Table 3-40) Liver function

Group	**GOT U/L**		**GPT U/L**		**Alkaline PhosphataseU/L**	
	Day 0	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**
Control (D.W.)	135.8±2.1	136.0±2.5	66.4±1.88	66.8±0.3	299.8±17..55	295.2±16.9
C.arientinum 400 mg/kg	135.3±12.0	136.0±0.2	63±2.0	65±1.0	263±4.9	260±4.9
C.arientinum 200 mg/kg	135.66±2.0	135.66±2.1	63.3±2.1	63.66±5.2	263±4.8	260±4.9
C.verum 400mg/kg	118.0±3.4	117.3±1.4	61±1.5	60.6±9.2	219±4.0	220±3.9
C.verum 200 mg/kg	120.6±1.7	121±0.57	65.3±2.0	66.3±0.66	277±9.1	276.66±5.3
C.aurantifolin 400 mg/kg	129.3±1.4	130±0.9	61.6±2.9	61.3±1.8	254±4.1	256.3±3.2
C.aurantifolin 200 mg/kg	125.3±4.9	126.3±2.2	63±2.5	64.3±2.9	223±12.8	223.3±13.6

(Data are expressed in mean± standard error of mean)

(Table 3-41) Kidney function

Group	Creatinine mg/dl		Urea mg/dl		Na mmol/L		K mmol/L	
	Day 0	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**
Control(D.W.)	0.58±.08	0.56±.03	39.4±3.2	40.4±2.5	140.2±1.6	140.8±1.8	5.7±0.37	5.4±0.24
C.arientinum 400 mg/kg	0.80±.08	0.76±.05	41.3±1.8	40.3±3	142.6±.34	142.3±1.5	6.20±0.29	6.30±0.07
C.arientinum 200 mg/kg	0.93±.08	0.90±.05	45±1.7	43±4.0	146.3±.33	146.6±1.4	5.5±0.29	5.7±0.08
C.verum 400 mg/kg	0.63±.12	0.6±.05	59.3±2.9	58.6±2.9	147.3±.33	146.3±4	5.2±0.14	5.4±0.32
C.verum 200 mg/kg	0.96±.03	0.93±.08	44±1.7	43±2.3	146.3±.8	145±.5	6.0±0.2	6.2±0.26
C.aurantifolin 400 mg/kg	0.93±.08	0.97±.05	45±1.7	43±4	147±.33	146.6±1.4	5.5±0.29	5.7±0.08
C.aurantifolin 200 mg/kg	0.96±.03	0.93±.03	46.66±1.7	44.3±8.1	144.3±1.7	143±2.6	5.20±0.08	5.50±0.057

Data are expressed in mean± standard error of mean

Table (3- 42) Haematology

Group	WBCs (Cells/cmm^3)		RBCs (Cells/cmm^3)		HGB (g/dl)	
	Day0	Day21	Day 0	Day21	Day 0	Day21
Control (D.W)	5.8±0.4	6.0±3	5.8±0.9	6.0±0.1	11.0±1	11.5±0.5
C.arientinum(400mg/kg)	7.3±0.1	7.1±2.	6.0±.01	6.3±0.3	11.3±.8	11.0±0.5
C.arientinum(200mg/kg)	5.0±0.4	5.2±1	9.7±3.2	9.9±0.1	10.7±.8	10.3±0.5
C.verum (400mg/kg)	5.6±0.2	5.8±4	6.0±0.3	5.9±0.2	10.2±.6	10.6±0.2
C.verum (200mg/kg)	4.6±0.1	4.8±2	6.2±.15	5.9±0.3	11.4±2.	11.5±0.1
C.aurantifolin(400mg/kg	6.2 ±3	6.2±3	10.5±.3	10.1±.4	10.4±0.5	10.8±0.6
C.aurantifolin(200mg/kg	2.7 ±.1	3.0±3.	5.2±0.2	5.1±0.4	10.8±.5	10.6±0.6

Table (3-43) Histopathology

Histopat-hological Findings	Control N=10		*C.arientinum* N=10		*C.verum* N=10		C.aurantifolin N=10	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Normal hepatocytes	+	+	+	+	+	+	+	+
Fibrosis	-	-	-	-	-	-	-	-
Cirrhosis	-	-	-	-	-	-	-	-
Fatty infilteration	-	-	-	-	-	-	-	-
Focal necrosis	-	-	-	-	-	-	-	-

Chapter four

4-Discussion

Plants have already been utilizable sources of drugs and many of the currently available drugs; have been directly or indirectly extracted from plants. In accordance to the recommendations of the WHO Expert Committee, on diabetes mellitus, it is important to investigate the hypoglycaemic action for plants which were traditionally used in traditional medicine (Alarcon., *et al* 1998).

Some rural old men and ladies, termed specialists, treat diabetes by some medicinal plants and they believe that traditional medicines are better, permanent cure for many diseases. So knowledge, accordingly, continue to provide the building blocks for the development of traditional medicine (Rahman and Zaman 1989).

The limited efficacy and the draw back of the currently used hypoglycemic agents prompted the scientists world-wide to search for more effective phytomedicenes. (Rahman and Zaman 1989).

More than 1200 species of plants have been used ethno-pharmacologically or experimentally to treat symptoms of diabetes mellitus. They represent more than 725 genera in 183 **families**. The most frequently sited families are *Asteraceae, Fabaceae, Poaceae*, *Laminaceae* and *Liliaceae*. According to the taxonomy of Elghazali., *et al* (1999) *C.arientinum* belongs to the family *Fabaceae C.verum* belongs to *Lauraceae* and *C.aurantifolin* belongs to the family *Rutaceae.*

In this study, **phytochemical screening** of *Cicer arientinum* revealed presence of sterol, alkaloids, saponin, cyanogenic glycosides and coumarins; in addition (Elghazali., *et al* 1999) reported presence of volatile oil, amino acids and starch in *C.arientinum*. Phytochemical screening of *Cinnamomum verum* revealed presence of triterpens, alkaloids, tannin and saponin, besides these (Elghazali., *et al* 1999) declared presence of volatile oil, cinnamic aldehyde as well as terpenoids.

Phytochemical screening of *Citrus aurantifolin* resulted in presence of sterols, alkaloids, flavonoids, saponin, cyanogenic glycosides and coumarins, (Elghazali ., *et al* 1999) added presence of volatile oil, hesperidin, vitamin B, vitamin C, and potassium and calcium citrate. On the basis of the above evidences it is possible that the presence of alkaloids, saponnins, sterols, volatile oils, glycosides, coumarins and flavonoides were responsible for the observed anti diabetic effect of *C.arientinum, C.verum* and *C.aurantifólin.* These findings **agree with** Grover., *et al* (2002) who reported that the biologically active components of plants with hypoglycaemic action include; flavonoides, alkaloids, glycosides, polysaccharides, peptidoglycans, steroids and terpenoides. The results of this study also agree with the findings of Baskaran ., *et al* (1990) who related the hypoglycaemic effect of *Gymnema sylvestre* to the presence of saponins, Ghosal., *et al* (1974) and Shani *et al*., (1974) who related the hypoglycaemic effect of *Trigonella,* to the presence of coumarins, De Sousa, who related the hypoglycaemic activity of *Bauhinia forficata* to the presence of flavonoids and (Lotlikar., *et al* l996) who related the hypoglycaemic effect of *M.charantia* and *M.foetida*, to the presence of glycosides.

The findings of this research **disagree with** the findings of Hou., *et al* (2003) who related the hypoglycaemic activity of *Lactuca indica* to the presence of latucain C (3) and lactucaside.

The present study was an attempt to investigate the **hypoglycaemic** and anti –diabetic effects of three medicinal plants used in folk medicine for the treatment of diabetes mellitus. These plants were *Cicer arientinum*, *Cinnamomum verum* and *Citrus aurantifolin*.

As shown in tables (3-3) and (3-6) the two doses (400 and 200 mg/kg) of both extracts of C.arientinum, showed a significant **glucose** reduction ($P<0.001$) and ($P<0.05$) respectively after extract administration to

hyperglycaemic rats. The hypoglycaemic effect of both extracts, was greater than that produced by glibenclamide. In **diabetic rats**, both doses (400 mg/kg and 200 mg/kg) of the aqueous extract, revealed significant hypoglycaemic effect (P<0.05) and (P<0.001) respectively. Both doses of the methanolic extract, revealed a slower but highly significant (P<0.001) hypoglycaemic effect. Both extracts recorded glucose – lowering effect greater than that caused by glibencamide and insulin.

Regarding the hypoglycaemic effect of ***C.verum*** in hyperglycaemic rats**,** the both doses of both extracts significant lowering effect (P<0.001) since the first hour post dose administration as shown in tables (3-9) and (3-12).In diabetic rats, dose (400 mg/kg) of the aqueous extract reduced blood glucose level significantly (P<0.05) (P<0.001), 8 and 12 hours post dosing respectively. Neither glibenclamide nor insulin reduced blood glucose level of diabetic rats as shown in table (3-27) Dose (200 mg/kg) of the aqueous extract and both doses of the methanolic extract, exerted significant glucose reduction (P<0. 001) after 12 hours as demonstrated in table (3-30).

In studying the hypoglycaemic effect of ***C.aurantifolin***, doses (400 and 200 mg/kg) of the aqueous extract reduced blood glucose of hyperglycaemic rats significantly (P<0.05), (P<0. 001) and (P<0. 001) at hours 1, 2 and 4 post dosing respectively as shown in table (3-15). Regarding the methanolic extract, dose (400 mg/kg) resulted in a significant reduction in blood glucose (P<0. 001) and (P<0.05), one and two hours respectively post administration of the extract. Dose (200 mg/kg) showed a significant lowering of blood sugar (P<0.05), one hour after administration of extract as shown in table (3-18)

In diabetic rats the two doses of the aqueous extract reduced blood glucose significantly (P<0.05), (P<0.001), 4 and 8 hours after extract administration respectively (table 3-33), while both doses of the

methanolic extract showed a significant hypoglycaemic effect (P<0.05) 12 hours post dosing. Neither the reference drugs glibenclamide nor insulin reduced blood glucose, as shown in table (3-36).

Many **other hypoglycaemic plants** were studied by different authors. In a study to determine the mechanism of the hypoglycemic activity of the aqueous extract of *Retama Ractam* (RR) in normal and streptocotocin induced diabetic rats, dose 10mg /kg, produced a significant decrease in blood. glucose level of normal rats and even more marked decrease in diabetic rats and a change in plasma insulin concentration after (RR) treatment (Mohamed, 2003).

The aqueous extracts of *Fraxinus excelsior* (FE) seeds and *Silybum Marionum* (SM) aerial part, were investigated for their hypoglycaemic effect in normal and Streptozotocin (STZ) diabetic rats. After 15 daily doses of oral administration of the aqueous extracts (2o mg/kg), a significant decrease of blood glucose level was produced in both normal and STZ diabetic rats. In addition no change in insulin level in plasma was observed. We conclude that aqueous extracts of FE and SM exhibit potent hypoglycemic and anti-hyperglycemic activities in normal and STZ rats, respectively, without affecting plasma insulin concentration. (Maghranim, *et al.*, 2004).

The leaf alcohol extract of the plant *Annona squamosa* was investigated for its anti-diabetic activity in diabetic rats. The findings showed the significant anti diabetic potential of the extract in monitoring the diabetic condition in diabetic rats (Annie., *et al* 2004).

The effect of the extract of *Origanum Vulagare* leaves on blood glucose levels was investigated in normal and streptozotocin induced diabetic rats. There was a significant decrease ($p < 0.05$) in blood glucose, after single oral administration for 15 days of repeated single daily doses of the aqueous extract (20 mg/kg). A significant decrease on blood glucose level

in STZ diabetic rats was observed. The aqueous extract of *Origanum Vulagare* exhibited an anti-hyperglycemic activity in STZ rats without affecting plasma insulin concentration. (Lemnadri *et al*, 2004).

The target plants of this present study are also subjected to investigation of their effects on **cholesterol and triglycerides**. Both doses of the aqueous extract of ***C.arientinum*** showed a persistent significant reduction ($P<0.05$) on blood cholestrol of **hyperglycaemic rats** at the first and second hours post extract administration. The results are shown in table (3-4), while both doses of the methanolic extract lowered the level of blood cholestrol of hyperglycaemic rats significantly ($P<0.001$) since the first hour of extract administration. The effect persisted throughout the experiment as shown in table (3-7). Similar to glibenclamide, and insulin, neither the aqueous (table 3-22) nor the methanolic (3-25) extracts of *C.arientinum* exerted a hypocholesterolaemic effect in diabetic rats.

With respect to ***C.verum*,**neither the aqueous nor the methanolic extracts produced significant reductions on the levels of blood cholesterol of hyperglycaemic as well as on diabetic rats as indicated in tables (3-10), (3-13), (3-28) and (3-31).

Both doses of the aqueous extract of ***C.aurantifolibn*** reduced blood cholesterol of hyperglycaemic rats significantly ($P<0.001$) 1 hour post dosing as shown in table (3-16).Dose 200 mg/kg revealed a significant hypocholesterolaemic effect ($P<0.05$) as demonstratd in table (3-19).In diabetic rats,similar to glibenclamide and insulin,neither the aqueous nor the methanolic extracts reduced blood cholesterol as shown in tables(3-34) and (3-37) respectively.

Concerning the effect of these plants on the level of blood **triglycerides** of **hyperglycaemic rats,** the two doses of the aqueous extract of ***C.arientinum*,** expressed a significant reduction ($P<0.05$) two hours post

dosing as compared to the control The results are shown in table (3-5). Both doses of the methanolic extract lowered the level of triglycerides significantly (P<0.001) since the first hour of extract administration. The effect persisted throughout the experiment as shown in table (3-8).

In **diabetic rats**, dose (400 mg/kg) of the aqueous as well as the methanolic extracts of C.arientinum reduced the level of blood triglycerides significantly (P<0.001), twelve hours after extract.administration while dose 200mg/kg showed a more persistent significant lowering effect (P<0.05), (P<0.05) and (P<0.001) respectively 4, 8 and 12 hours post dosing. The results are shown in table (3-23). Similar to glibenclamide, dose 200 mg/kg showed no lowering effect on blood triglycerides, while Insulin reduced blood triglycerides significantly (P<0.001) 4, 8, and 12 hours post administration. Table (3-26).

In studying the effect of ***C.verum*** on the level of blood triglycerides of **hyperglycaemic rats**, the results revealed that none of the two doses of the aqueous extract, produced a significant lowering effect on the level of blood triglycerides as indicated in table (3-11), while the two doses of the methanolic extract produced a significant reduction (P<0.05) 2 and 4 hours post dosing, as compared to the control (table 3-14).In **diabetic rats,** both doses of the aqueous extract of *C.verum* produced a significant lowering effect (P<0. 001). on the level of blood triglycerides of diabetic rats 8 and 12 hours post dosing. The results are illustrated on (table 3-29). The methanolic extract, as well as the reference drug (insulin) showed a significant lowering effect (P<0. 001) on the level of blood triglycerides, twelve hours post dosing, as shown in table (3-32).

In studying the effect of ***C.aurantifolin*** on the level of blood triglycerides of **hyperglycaemic rats**, dose (400 mg/kg) of the aqueous extract, revealed a significant reduction (P<0. 001) 2 and 4 hours post dose

administration as shown in table (3-17), while the methanolic extract showed no significant reduction as illustrated in table (3-20). **In diabetic rats,** both doses of the aqueous extract of *C.aurantifolin* as well as the reference drug (insulin) reduced the level of blood triglycerides significantly (P<0. 001) since the 4th hour, while glibenclamide showed no effect. The results are shown in table (3-35). The two doses of the methanolic extract of *C.aurantifolin* reduced the level of blood triglycerides of diabetic rats significantly (P<0.05), (P<0. 001) 8 and 12 hours post extract administration. The reference drug insulin produced a highly significant reduction (P<0. 001).since the 4th hour and the effect persisted till the 12th hour. Glibenclamide showed no effect. The results are shown in table (3-38).

Other studies on the hypolipidaemic effects of hypoglycaemic plants are conducted worldwide. Oral administration of *Trigonella foenum-graecum* seeds for 5 successive days to normal rabbits resulted in a significant reduction of serum glucose concentration and showed no effect on cholesterol and triglycerides. In diabetic rabbits, administration of the same extract of the seeds of *Trigonella foenum-graecum*, significantly reduced serum glucose and cholesterol (Al-Hussary 1993).

A study performed by Shiva 1998 revealed that *Trigonella foenum gracecum* lowers blood cholesterol and triglycerides. A study conducted by Agrawal., et al 1996, verified that *Ocimum sanctum*, reduced blood glucose, uric acid, total amino acids, cholesterol triglycerides and phospholipids.

The suggested **mode of action** for the study plants is inhibiting gluconeogenesis and facilitating glucose absorption by the cells i.e. increase glucose metabolism and trigger release of insulin. Leung and Foster, (1996) reported that ***C.verum*** has shown a strong lipolytic action (hydrolyze fats). Bruneton, 1995 reported that the essential oils in

C.verum have demonstrated antifungal, antiviral, bactericidal and larvicidal actions.

Many **previous studies** were performed to investigate the **mode of action** of some hypoglycaemic plants. The mechanism may differ from one plant to another but the net effect is more or less the same. *Ptercarpus marsupium* has been proved for their regenerative/repair activity of B-islets of pancreas. It has insulin like property in cases of newly diagnosed Non-Insulin Dependent Diabetes Mellitus (Gupta 1983) and (Shah 1967). The herbal therapy *Gymnema Sylvestra* (gumar) appears to bring about glucose homeostasis through increased serum insulin levels provided by repair/regeneration of endocrine pancreas. *G. Sylvestra* treatment helped to maintain the weight of pancreas; number of beta- cells in diabetic subjects.Gymremic acid had lowering effects in blood glucose level (Murakami., *et al* 1996).

Aegel marmaelos exerts a hypoglycaemic effect, by improving digestion and reducing sugar and urea *Embilica aplicenel* (Indian gooseberry) produces a hypoglycaemic effect by stimulating pancreatic secretion of insulin (Upadhyay., *et al* 1996). Kohli and Singh (1993) found that *Eugenia jambiana* prevents pathological conversion of starch to glucose, thus reducing glucose level in blood. The flavonoids quercetin and myricetin have also been reported to be hypoglycemic(Rahman and Zaman 1989),but they are known to be potent inhibitors of protein tyrosine kinase the activity of which is essential in the post receptor – binding activity of insulin. Most hypoglycemic plant constituents, such as alkaloid, salicylic acid are also growth inhibitors and hypoglycemic agents (Geahlen ., *et al* 1989),The mode of action produced by the fruits, leaves and roots of *Momordica charantia* in reducing blood glucose, is supposed to be through increasing glucose uptake in the liver cells and by acting as plant insulin (Kavikumar *et al* 1997).

In studying the **toxicity** of the study plants, neither *C. arientinum* nor *C.veum* or *C.aurantifolin* showed any change in the levels of T.WBCs , T.RBCs and Hb as shown in table (3-39).Concerning liver function tests, neither *C.arientinum* nor *C.veum* or *C.aurantifolin* showed any change in the levels of GOT, GPT and ALP as shown in table (3-40). Concerning kidney function tests, neither *C.arientinum* nor *C.veum* or *C.aurantifolin* showed any change in the levels of Urea, Creatinine, Na and K as shown in table (3-41). None of the study plants showed any histopathological findings as shown in table (3 - 42).

Unlike our findings, organ lesions accompanied by leucopenia, anaemia and alterations in serum GOT, GPT and ALP activities and concentrations of total protein, albumin, urea, bilirubin and other serum constituents was reported in rats fed *Nerium oleander* leaves (Al – Yahya et al., 2000). Increased ALP activity was reported in chicken fed *Azadirachta indica* due to excretory dysfunction of the liver (Ibrahim, 1990). Graded doses of Nature Cure Bitters (*Hilleria latifolia*, *Citrus aurantifolia* and *Xylopia aethiopica*) were administered daily (100, 200 and 400 mg/kg) to rats for 28 days, a significant decrease in ALP and in total protein occurred in all groups with an elevated level of albumin and GPT (Stanley *et al*, 2000). *Guiera sengalensis* and *Ambrosia maritime*, which were used in folk medicine for treatment of diabetes mellitus, caused hyperplasia to the pancreas (Ahmed, 1987).

Conclusion

In this study, it was noticed that the three study plants *C.arientunum, C.verum* and *C.aurantifolin*, revealed significant hypoglycaemic effect in both type I and type II Diabetes mellitus. *Cinnanomum verum* was the most effective one. The onset of action for the three plants was earlier in type II diabetes mellitus. In type 11, all plants were more effective than the reference drug Glibenclamide, similarly in type I all plants showed a hypoglycaemic effect greater than that caused by the reference drug Insulin.

Regarding the effect of these plants in blood lipids, none of them affected the level of blood cholesterol in type I diabetic rats. *Cicer arientinum* was the most effective one followed by *Citrus aurantifolin*.
With respect to triglycerides, the methanolic extract of *C.arientinum* was the most effective one of the three study plants in reducing blood triglycerides in type II, while the aqueous extract of *C.aurantifolin* was highly effective in reducing blood triglycerides of diabetic rats. The effect of *Cinnanomum verum* was the least and was more pronounced in type I.

Regarding toxicity all three plants proved great safety in their continuous use according to their effects on liver functions, kidney functions and blood haemogram.Furthermore a dose of 10 g / kg was reached without any death in the experimental rats, which ensures great safety of the tested plants.

From the results of this study, it can be concluded that *C. arientinum, C.verum* and *C.aurantifolin* are highly effective and safe hypoglycaemic agents for both types of diabetes mellitus. Furthermore they act as moderate hypolipidaemic agents.

Recommendations

Sudan in a very rich country in medicinal herbal plants whose scientific knowledge is almost unknown, thus the following guidelines are recommended:

- Development of cultivation of indigenous medicinal plants.
- Development of well equipped laboratories for the proper assessment of different herbs including *C.arientinum, C.verum* and *C.aurantifolin.*
- Health education on beneficial effects of natural plants.
- Establishing more research centers for studying and training staff and students in herbal plants.

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Appendix